The effects of temperature, light and rainfall on the persistence of lindane, fenitrothion and permethrin when evaluated principally as stomach poisons against the desert locust (Schistocerca gregaria Forsk.).

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THE EFFECTS OF TEMPERATURE, LIGHT AND RAINFALL ON THE PERSISTENCE OF LINDANE, FENITROTHION AND PERMETHRIN WHEN EVALUATED PRINCIPALLY AS STOMACH POISONS AGAINST THE DESERT LOCUST (*SCHISTOCERCA GREGARIA* FORSK.)

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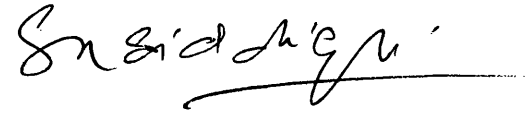

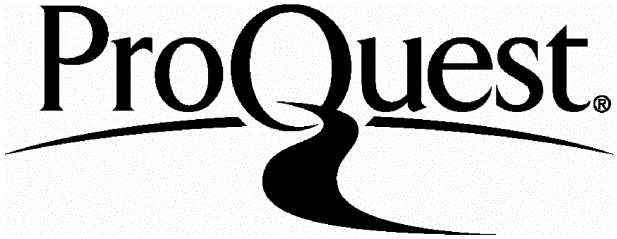

CONTENTS

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ABSTRACT

LD_{50} and LD_{90} values were determined by bioassay of topical and stomach poison applications for lindane, fenitrothion and permethrin. At equivalent dosage rates fenitrothion was generally the most and permethrin the least, effective. Permethrin and fenitrothion were more toxic as contact poisons whereas lindane was more effective as a stomach poison.

Using LD_{50} and LD_{90} values and sucrose treated tissue paper as a substrate, the persistence of three insecticides as stomach poison was determined at various temperature regimes with light excluded. Both lindane dosages had a short persistence (3 - 7 days) at $5^{o}C$ and $15^{o}C$; fenitrothion had a similar persistence at $15^{o}C$ and a moderate persistence at $5^{o}C$ (LD_{50} for 7 - 14 days and LD_{90} for 14 days) but permethrin retained its toxicity for at least 21 days at $5^{o}C$, $15^{o}C$ and $30^{o}C$. No additional degradation was obtained at $5^{o}C$ with a continuous light intensity of 2415.6 lux for any of the insecticides and permethrin retained its toxicity for at least 56 days.

Persistence of fenitrothion (LD_{90}) and permethrin (LD_{50}) were evaluated on wheat, privet and Brussels sprout plants growing under the same light and temperature regimes referred to above. The plants had no effect on the persistence of fenitrothion. Permethrin retained its toxicity for more than 40 days but slightly higher levels of activity were retained on wheat than on the other two plants.

Rain fastness on growing plants was only evaluated for permethrin; there was little loss in wheat, a greater loss on privet and Brussels

1. INTRODUCTION

Agriculture is the backbone of most developing countries of the world. About eightyper cent of the national economy of these countries depends on agricultural products as food or raw materials for industry. The occurrence of pests and diseases is a constant threat to the agricultural economy as a whole.

Because of great advancement in medical technology, less epidemic diseases occur, more children are born and more of them survive. The net result of all this is an increase in population. Ultimately, to feed all these people more crops must be grown, particularly the grain crops such as rice, wheat, barley, maize and sorghum. Besides the food products, agriculture is also the main source of fibrous raw material, like cotton and sugarcane for industrial purposes. If man has to survive, he has to compete with so many creatures in this world, especially insect pests and diseases, which are a constant threat to agricultural crops.

Minimum estimated losses due to the attack of insect pests and disease every year in Pakistan have been assessed as US $ 368 million (Huque, 1973).

In Pakistan, agricultural crops are attacked by various insect pests every year, but *Schistocerca gregaria* (Forsk), the desert locust is considered to be one of the major pests.

Haskell (1970) reports that the first locust plague was observed

eight or nine thousand years ago, when man started growing wild species of plants as food. Locusts have been defined as grass hoppers having the capability of changing their habits and behaviour when they occur in large numbers. At high population densities they become gregarious in habit staying together in dense groups, which are known as swarms. The swarm is composed of adults and bands of hoppers. The 'hopper' is a wingless young stage. When all the locusts become fully winged the swarms can migrate over long distances; this characteristic and the gregarious habit are the outstanding features that distinguish locusts from grass hoppers. When locusts occur in small numbers and live their individual lives like ordinary grass hoppers, they are referred to as being in the solitary phase.

The life cycle of all the species of locusts can be divided into three principal stages, namely egg, hopper, and adult. The length of these stages varies depending on the prevailing weather conditions. Adults of desert locusts (*S. gregaria)* are yellow in colour, males are smaller in size and are darker in colour. After copulation, the female lays eggs in moistened sand. Each egg pod contains 70-100 eggs which, depending on favourable weather conditions, take 14 days to hatch. The next stage consists of non flying hoppers, which are able to migrate by marching. The hoppers normally pass through five instars before becoming immature adults or fledgelings. During the fledgeling stage the locust can undertake long flights and in this stage most feeding occurs. So the total period from the laying of eggs of one generation to a similar stage in the next generation is estimated to be about four months under favourable conditions.

With the desert locust there is no single area from which locust plagues start. The swarms move between seasonal breeding areas with the dominant winds and there are certain areas in each country concerned where swarms and breeding can be expected at a particular season.

Desert locusts during the plague period may invade a total area of 11 million sq. miles (though not all at the same time); this is more than 20% of the total land surface of the world. The invasion area contains a great variety of climatic conditions, soil types and forms of vegetation. Breeding of the desert locust takes place during the rainy season. Breeding only takes place below 5,000 feet sea level, although locust swarms have been recorded 10,000 feet above sea level.

Locusts cause damage by eating leaves, flowers, fruits, seeds, bark and growing point,and also by spoiling plants with their excreta. The effect of damage varies according to the stage of growth of the plants or crops and the variety; for example, on sugar-cane crops in Pakistan, the damage is greatest when it occurs during the first four months of cane growth.

The desert locust (*Schistocerca gregaria*) causes severe damage to the agricultural crops of many countries in the world. In 1931, locusts destroyed 20% of the subsistence crops and caused near famine in the world. In S. Morocco, citrus crops worth US $ 14 million were destroyed within six weeks. Crops worth £400,000 were destroyed by locusts during 1926-1934 in India. Cereals weighing 167,000 tonnes, sufficient to feed one million people in Ethiopia for one year, were destroyed by locusts during the year 1958. The cotton crop, valued

at £300,000 on 10,000 acres of land was destroyed by locusts in India in 1962. Besides causing direct losses, locusts can severely damage range lands and so reduce the production of meat from stock.

The damage to the crops is caused by all the active developmental stages of the locusts. Analysis of 2,000 records of desert locust damage shows that 8% of the damage is caused by hoppers, 69% by fledgelings and maturing swarms, and 23% by matured swarms. The low damage by the hoppers is considered to be due to the fact that the breeding areas are mostly outside the main crop areas.

The estimated average annual loss caused by locusts in general has been given by Cramer (1967), and Gunn (1960). In the world as a whole, the estimated average annual loss for the damage between 1925-1934 was £8.3 million and the cost of control measure was £1.3 million. North America accounted for an annual average damage of £5.9 million. In Africa, average annual damage by locusts was £0.5 million, as compared to the cost of control, which was £0.4 million. The cost to 64 countries of the world for controlling the desert locust alone in 1956 was £5 million.

The discovery of organic pesticides provided man with new and powerful weapons for his incessant war against insect pests and diseases. Today their use is recognised throughout the world as an effective, relatively simple and quick method of pest control. Without chemical control man's crops would be ravaged by disease and insect pests, and severe loss of food production would undoubtedly occur. One can point to examples which show this to be so. Today potato growers can

produce blight-free crops, in contrast to the catastrophic situation in 1840 when *Phytophthora infestans* damage led directly to the Irish famine. Cotton is an important source of oil and protein as well as fibre, but production can be drastically reduced by insect damage, so much that over a third of insecticides used in agriculture are applied to this crop (Matthews, 1979).

Chemical control is still the only effective method of controlling most insect pests and diseases, despite intensive research into alternative methods. Pesticides remain our most powerful tool in pest management in spite of recent popular pressure to have their use curtailed. Higher standards of living by an ever increasing human population will undoubtedly mean increased use of pesticides. This situation will be particularly true in the developing countries, which currently use less than 10% of the total world production. Southwood (1977) pointed out that pesticides are a valuable resource and must be used wisely if we are to reduce the amount of chemical applied and the number of applications. In doing this we would decrease selection pressure for resistance, prolong the useful life of each pesticide and reduce environmental contamination and residues in food.

There are several methods of applying insecticides for locust control, such as baiting, dusting, spraying (ground as well as aerial), barrier or lattice spraying (Uvarov, 1977; Rainey, 1974).

The best way of controlling locusts is to prevent plagues rather than to suppress them after swarming has begun. This method has been successful with two species of locusts, but not with the desert locust

as there is no permanent or regular plague source which can be checked. Therefore the problem of swarms occurring or developing in many different places and countries still exists. The guiding principle for controlling locusts is to kill the maximum number; this means target selection at the time of widespread infestation. Therefore, the aim is to attack the egg fields either by direct contact or before hatching by applying appropriate residual contact insecticides. The next target is the nymphal bands, which are relatively static and so can also be attacked by direct contact application of insecticides or by lattice spraying of the field. Ground control should be adequate provided the infested areas are accessible to vehicles and other equipment. Adult control is the last resort and is carried out by aerial spraying, with direct contact application, either to roosting locusts or as a 'spray curtain' against flying locusts. Methods of locust control have improved over the last three or four decades, progressing from the older conventional methods such as baiting and dusting to the use of modern techniques, including ULV spraying utilizing both aerial and ground equipment. Such methods have been described by MacCuaig (1963, 1969) and Matthews (1979).

Chemicals used extensively for locust control include both Organochlorinated and Organophosphorus compounds such as aldrin, dieldrin, lindane, fenitrothion, and malathion. Dieldrin has proved to be highly toxic to locusts both as a contact and a stomach poison. It is known that dieldrin is highly stable and its use has been criticised because of its extremely long persistence in the environment. This has led the Swedish government to ban completely its use. However, some persistence is desirable in lattice spraying and when applications are made against

slow moving hopper bands in open desert. But the extreme persistence of dieldrin is never really necessary. At other times for example against flying locusts the persistence is not required. The same is true of general agriculture - sometimes persistence is needed and at other times, for example, when insecticides are applied to crops close to harvest persistence is undesirable, because of the danger of imparting toxic residues to edible parts of the crop.

Generally public opinion equates pollution and persistence together and considers that persistent insecticides are undesirable. However laudible is this comment, any replacement of persistent insecticides by non-persistent ones (even if available) would increase the number of application of pesticides, so raising the cost of food and fibre crop production.

Economics therefore dictate that persistence is likely to remain as an important feature of insecticides and is a factor that needs to be determined. Knowledge of the persistence of a particular compound under various environmental conditions allows the farmer to know when to spray again, if necessary, and whether the produce is safe for human consumption or for cattle to forage on the sprayed vegetation.

The aim of this research project was to achieve the following objectives:-

1. To evaluate the persistence of some existing or candidate insecticides against locusts.
2. To relate persistence to various environmental factors likely to be encountered in the field (especially in tropical areas).

3. To evaluate the stomach poison persistence of the insecticides rather than assessing the contact effect which is usually investigated. To do this a bioassay technique was considered essential.
4. Perhaps to find a replacement for the highly persistent dieldrin.

Determination of Pesticide Residues

Pesticide residues can be determined by a) bioassay and b) chemical methods. Busvine (1971), gives examples of bioassay as contact poisoning or topical application, stomach poisoning tests and dry film tests. Under chemical methods he includes gas liquid chromatography and mass spectrographic techniques, but these techniques are costly to use in developing countries like Pakistan, so a simple technique that of colorimetric determination seems more applicable.

Draper (1976) describes the technique of colorimetry as the measurement of the absorption of light by a coloured solution. In optical methods of analysis, it is the absorption of light by molecules which is measured.

Several techniques have been developed for the micro determination of pesticides, especially for the organophosphates. In the present investigation, the technique described by Getz and Watts (1964) was employed.

Previous Work on Persistence

Pesticides occur in detectable amounts throughout the environment

in virtually all inhabited areas of the world and in some, if not all, of the uninhabited portions. Pesticides are introduced into the environment in a variety of ways, including direct application in agriculture, in forest pest control, and for control of pests affecting human health. The amount of synthetic chemicals, including various kinds of pesticides, produced during the last 20 years are relatively large. Every year millions of pounds of pesticides are produced throughout the world. Some of these pesticides are highly persistent and toxic causing residues which endanger human and animal life.

The persistence and ultimate fate of pesticides in the food, soil, water and air of man's environment is affected by such interrelated factors as volatility, solubility, ultra-violet irradiation, surface adsorption, systemic action, hydrolysis, chemical rearrangements etc. The combined weathering action of rain, wind, temperature, and humidity exerts considerable influence on the exposed pesticide breakdown.

Persistence is something ambiguous. It is conveniently encountered in two distinct forms, one of which is undesirable and one of which is sometimes useful. The term 'persistent' when used in a negative sense characterizes both something static, that is when a compound is found at a location or at a time where or when it is not expected, as well as something dynamic, in other words when a compound undergoes degradation at a slower rate than is seemingly necessary or desirable. When the term is used in a positive sense, it is understood to be synonymous with residual activity, thus indicating that a compound possesses the effect ascribed to it not only a priori but is also capable of retaining it for a certain period.

Without doubt, persistence is a most important factor, but whether it is desired or undesired, will depend upon how a particular pesticide is expected to perform in commercial application. In agriculture, a possible reference period would be the growing season. Here the question of persistence is of practical importance. Pesticidal compounds which continue to remain active beyond the growing season may cause damage to following crops grown in rotation. A pesticide persists in order to act for a desired period. This property from the agricultural aspect is positive, and should be termed a residual effect (Frehse, 1976).

A lot of work has been done on the persistence of pesticides in the soil. The fate of pesticides in the soil depends on their chemical structure, the soil type, soil moisture, micro-organism content and cultural practices. Pesticides usually find their way into surface and ground waters as a result of agricultural land drainage or industrial waste discharge. Soils are directly contaminated by crop spraying for insect control or by soil treatment with insecticides.

Insecticides belonging to the chlorinated hydrocarbon group, such as DDT, aldrin, dieldrin, and heptachlor, are more persistent in the soil than those belonging to the organophosphorus group of pesticides. Large differences exist in each group of chemicals, whose half lives in soil range from several years to days.

Soil temperatures have remarkable effects on the rate of loss of insecticides. The temperature affects both the loss through volatilization as well as the breakdown of the insecticides by biological and chemical factors (Lichtenstein and Schulz, 1960).

Nasim *et al.* (1971), carried out random examination of six samples of soil in Pakistan and found that all the samples were contaminated with organochlorinated insecticides and contained 1-3 ppm of DDT. In addition three samples contained 0.1 - 0.5 ppm of lindane and the other three contained approximately 0.5 ppm of dieldrin.

Less work has been done on the persistence of insecticides on vegetation —hence one of the reasons for the present research. However, previous work suggests that the following factors are relevant:-

1) Temperature
2) Moisture
3) Volatilization
4) Light - especially UV light
5) Chlorophyll activity, plant tissue surface
6) Hydrogen ion (pH)
7) Intrinsic qualities of the insecticide and formulation used i.e. emulsifiable concentrations, or in oil.

Vaporization or volatilization of an insecticide from the applied surfaces is controlled by many variables, particularly the nature of the insecticide, solubility, degree of absorption and above all the atmospheric temperature (Hawker, 1972; Spencer *et al.*, 1969). Initially there are two factors responsible for the volatilization of a deposit, firstly the vapour pressure of the pesticide and secondly the rate of movement away from the evaporating surface. Close to the evaporating surface there is relatively no air movement and the vaporized substance is transported from the surface through this stagnant air layer only by

molecular diffusion. Diffusion away from the surface is related to the vapour pressure of the insecticide, its molecular weight and the temperature which influences both vapour pressure and the diffusion process itself (Hartley, 1969). The breakdown of an insecticide is a completely different process from volatilization. With chemical degradation the insecticide is chemically broken into toxic or non-toxic chemical compounds, due to chemical or physical processes. Vapour pressure is normally very low and so the movement away from the evaporating surface is limited. So factors affecting the toxicity of insecticides to individual locusts can be summarized as follows:-

1) Temperature
2) Age
3) Instar and age within instar
4) Weight
5) Humidity
6) Day-length
7) Speed of action
8) Feeding regime
9) Contact, stomach, injection effects

For these reasons, in the present work, factors like temperature, age, instar and age within instar, humidity, day-length, speed of action and feeding regimes, were standardised and experimental variation of factors, such as weight, and contact and stomach action of the insecticides were investigated.

The extensive usage of organochlorinated insecticides, during the

past three or four decades has caused international concern, as this group of pesticides has proved to be persistent. Persistence and accumulation in the ecosystem may lead to the development of resistance in insects. Resistance is undoubtedly one of the most undesirable side effects of pesticide usage. The subject has been comprehensively reviewed by Brown (1971) and Perry and Agosin (1973).

That locusts are able to develop resistance has been demonstrated by Shafi (1974) with *Schistocerca gregaria*, who found that the resistance level had risen more than three fold by the F6 generation with lindane, two and a half times with fenitrothion in the F8 generation and the LD_{50} rose from 1.54 to 2.46 µg/g with aprocarb by the F4 generation.

Busvine (1971) reports that the toxicity of insecticides is affected by several factors. Some of these are intrinsic such as age, instar and weight of the target species. Others are extrinsic caused by changes in the environment such as temperature, humidity, and length of day. Many extrinsic factors are known to affect the results of insect toxicity tests. Regardless of the technique employed, factors such as temperature and light directly affect the mortality of insects exposed to insecticides.

Temperature is probably the most important variable directly affecting insects during the period of exposure to insecticides. Temperature also affects the action of insecticides. Most insecticides are more toxic at moderate temperatures than at temperature extremes.

Guthrie (1950) found that DDT, pyrethrum, and lindane were more effective against the German cockroach *Blatella germanica* at 14.5°C

than 32°C, conversely he found that aldrin and dieldrin were more effective at 32°C, than at 14.5°C.

Hoffman and Lindquist (1949) determined that faster knock down and higher mortality of houseflies occurred at 21.1°C than at 26.5°C, using DDT, DDD and methoxychlor. The reverse was true of heptachlor, paration, chlordane and toxaphene.

Hoffman *et al.* (1949) found that more sheep ticks *(Melanophagus ovinus)* (L) in treated wool were killed at 21.1°C than at 26.5°C when exposed to DDT, DDD, or methoxychlor. The reverse occurred with toxaphene, benzene, hexachloride and chlordane.

Dustan (1947) showed that mortality due to DDT by both stomach and contact action declined as the temperature was raised from 15.5°C to 35°C. The studies were carried out on the diamond-black moth, *Plutella maculipennis.*

Studies on the effect of temperatures of 15°C and 35°C on the toxicity of DDT to the American cockroach *(Periplaneta americana)*, showed that DDT shows a negative temperature coefficient, it being more lethal at 15°C than at 35°C (Vinson and Kearns, 1952).

Toxaphene, dieldrin, and EPN retained their toxicity to boll weevils at a high temperature and low humidity better than parathion, malathion, endrin, or aldrin, as shown by Gaines and Mistri (1952).

The uptake of DDT by houseflies increased with the time of contact. The addition of a small quantity of moisture to a DDT deposit shortened

the toxic induction period and produced a measurable decrease in the LD_{50}. Natural light as compared to darkness made flies more sensitive to DDT (Laug, 1946).

The behaviour of mosquitoes is affected by DDT, which causes irritability and induces flight, sometimes enabling them to escape from toxic residues. Kaschef (1970), conducted a series of investigations on *Anopheles labrianchiae atroparvus, A. gautiae species B and A. phorensis* to find out the effects of temperature on irritability caused by DDT or its analogues methoxychlor and DDD. The flight activity increased in both species with the increase of temperature, but was reduced at 32°C. The DDT resistant species *Anopheles phorensis* showed less irritability.

Post treatment coefficient tests were conducted on *Locusta migratoria migratorides,* using trichlorophon, parathion, malathion, malaoxon, diazinon, paraoxon, aldrin, dieldrin, lindane, DDT, carbaryl, and pyrethrins at 15°C, 27°C and 35°C by Rai (1967). It was observed that at the end point of tests, the value of the post treatment temperature coefficient (PTC) of the insecticides depends on their positive or negative rates of toxicant balance formation *in vivo* and rates of penetration. The decrease of positive PTC and the increase in the extent of negative PTC with time after treatment, are due to greater speed of action of insecticides at higher temperatures. For topical application of DDT and injection of trichlorophon, the dose effect is greater at 27°C and 35°C, while with the rest of insecticides, the dose effect is not significantly different at the three temperatures.

Fisher and Hansell (1964), made a study on kelthane MF, an acaricide, to investigate the post treatment temperature coefficient toxicity to *Tetranychus telarius;* it showed a positive coefficient on the test mites, the mortality increasing 1.2% for each degree rise in temperature.

Breakdown of DDT is not considered to be through the effect of temperature, as the rate of decomposition did not increase in the summer period (25 - 35) weeks, with its higher temperature (Gallahar and Evans, 1961). But there is evidence of disappearance of DDT from the soil at high temperature.

Toxicity tests on adult mosquitoes showed that the LD_{50} of dieldrin, carbaryl, and phosphoramidotheoate decreased and that of DDT increased between 20°C and 30°C (Hadaway and Barlow, 1963).

Concentrations of HCH in river water in Japan were determined by Ochiai and Hanya (1976) and results of their studies were based on a survey for a period of one year. Concentrations of HCH residues ranged from 5 - 577 ng/l of alpha HCH and from 5 - 234 ng/l of gamma HCH. The residual level was highest during the summer season.

Burgess and Sweetman (1949) made a laboratory study over three years on the residual property of DDT as influenced by temperature and moisture. The test insect was *Musca domestica* (L). The experiments were conducted in such a way that sunlight was avoided and indirect light of low intensity was used. They concluded that a high temperature of 37°C and a relative humidity of 60 - 75% reduced the toxicity of DDT to houseflies over a period of 39 months, whereas similar DDT-treated screens, held at 23°C, remained highly effective for the same

period. The loss of DDT residues was probably caused by volatilisation.

When a pesticide is applied to agricultural crops, it will be affected by different environmental conditions which may change its efficacy. Some pesticides, or their degradation products, may persist as residues detrimental to the other organisms within the ecosystem, therefore one of the foremost considerations in the development of a new insecticide for agricultural use is its possible effects on plants and beneficial organisms. The effect of sunlight on insecticides when applied to agricultural crops is interrelated with temperature and humidity.

Fleck (1949) found that DDT decomposition is catalyzed by ultraviolet light.

Studies by Lindquist *et al.* (1946) showed that both ultraviolet light and sunlight reduced the effectiveness of DDT to houseflies. The loss of DDT due to the effects of light varied with different formulations.

Aldrin and dieldrin are converted to their respective half-cage isomers of photo-aldrin and photo-dieldrin respectively due to sunlight (Rosen *et al.*, 1966). Similar chemical structures for photodieldrin were identified by Rosen *et al.* (1968) and Parson (1966).

Sunlight also converts endrin to its isomer, keto endrin (Rosen and Carey, 1968).

Rosen (1972) carried out his studies on the photodecomposition of abate, which is a larvicide. When abate is exposed to sunlight at a concentration of 600 ppm., in methanol solution, it was rapidly decomposed. The major initial product identified was abate-sulfoxide, which apparently further photolyzed rapidly to at least six other products. In addition, abatesulfone was formed either by further oxidation of abatesulfoxide or directly from the original compound, i.e. abate. Exposure of sulfone to sunlight for 9 days in methanol in a quartz flask resulted in no reaction, although exposure in the photosensitive solvent, acetone, for the same period led to extensive photodecomposition with the formation of a new unidentified compound.

Work on one hundred and forty one pesticides, using a paper chromatographic technique, was carried out by Mitchell (1961), to evaluate the effects of daylight, longwave ultraviolet light, and shortwave ultraviolet light. He concluded that light causes little or no change in the pesticides, or causes complete degradation, or it produces changes in the pesticides that lie between the two extremes.

Mitchell (1961) determined that dieldrin and aldrin were readily decomposed by ultraviolet light. Further studies showed that one ultraviolet decomposed product was similar to the product obtainable under natural conditions, by exposing dieldrin treated grass for several months (Roburn 1963). Other studies by Roburn showed that a single photo conversion product was obtained in yields of 7% after 3 weeks and 25% after two months by exposing dieldrin to sunlight. The same decomposed product was obtained by exposing dieldrin to a germicidal lamp for 48 hours; toxicity of this product is two times greater than

the parent compound to houseflies (*M.domestica* L.) and mosquitoes.

Laboratory studies by Crosby and Leitis (1973) showed that the hydrogen ion concentration can exert a strong influence on the photolysis of a pesticide. Under acidic conditions, photodecomposition was rapid and resulted primarily in polar products. At pH 11 the product profile was quite different.

The synthetic pyrethroid, SBP-1382, attracted the attention of Rosen (1972), because of the extensive use of this pyrethroid against the gypsy moth *Porthetria dispar*. Using the modified technique desdcribed by Chen and Casida (1969), he found that both the pyrethroid and its alcoholic moiety photodecomposed very rapidly, with no detectable parent material after 5 hours. The experiment was repeated on glass plates because of the apparent dark decomposition of SBP-1382 on silica gel. His findings were that there was a rapid decomposition of the irradiated material, but a thin layer chromatogram of the mixture on the glass was different than that from the silica gel irradiation.

There is evidence that chlorophyll activates the catalysation in photodegradation of pyrethroids (Glenn Jones, 1960).

Chen and Casida (1969) made a study on the photodecomposition of pyrethroids. They came to the conclusion that Pyrethrin I alletrin, phthalthrin, and dimethrin are each readily photolysed in the presence of air to at least eleven products, none of which has been identified. However, some of the changes occurring in the chrysanthemic acid have

been established.

Permethrin is 10 - 100 times more stable in light than previous pyrethroids as described by Elliott *et al.* (1973).

Frawley (1958) reports that the exposure of parathion to ultraviolet light gave rise to a mixture of compounds possessing greater *in vitro* anticholinesterase activity than parathion, but with lower toxicity to houseflies.

Payton (1953) demonstrated that the anticholinesterase activity of parathion is increased when exposed to UV light and sunlight.

Cook (1955) and Cook and Pugh (1957) indicated that the toxicity of parathion is decreased under UV light, but the *in vitro* anticholinesterase activity increased with the formation of more polar compounds.

Murai (1977) investigated the photodecomposition of edifenphos under UV light, using S-labelled compounds in the laboratory. Under catalysis by UV light, edifenphos was hydrolyzed to O-Ethyl S phenyl hydrogen phosphorothioalate, then ethyl dihydrogen phosphate and finally to phosphoric acid. The main step of photodecomposition was cleavage of the P-S bond at the initial stage of irradiation.

Effects of sunlight and ultraviolet light on the degradation of six N-methylcarbamate insecticides were observed by Crosby *et al.* (1965). They concluded that, with the exception of one inseticide, all the other five pesticides decomposed to give unidentified cholinesterase

inhibitors as well as other chemical compounds, both with sunlight and ultraviolet light.

The persistence of insecticides on crops is often evaluated under conditions that bear little relationship with those existing in the tropics with the result that field performance often fails to reach predicted standards.

Harrison *et al.* (1967), working on the persistence of some organochlorinated pesticides, evaluated the presence of both changed and unchanged pesticides and their conversion products on apple leaves in the field. They found that deposits resulting from the application of emulsifiable concentrates were more persistent than those from the same pesticide applied as dispersible powders. Dieldrin and DDT emulsifiable concentrates gave the most persistent deposits, residues taking eleven weeks to fall below 5% of the initial deposits. Aldrin, dieldrin, and endosulfan gave significant amounts of ultraviolet irradiation products.

Leaf analysis was undertaken to evaluate the persistence and degradation of dimethoate, a systemic insecticide, generally used for foliar application. Studies were made by Dauterman *et al.* (1960), on the leaves of corn, cotton, pea and potato plants, using radioactive dimethoate. They observed that the insecticide was readily absorbed and decomposed, both on the surface and inside the foliage. Phosphoric acid was the predominant identified product on near mature peas. The two identified products on the surface and inside of the leaf tissue were O,O-dimethyl S-carboxymethyl phosphorothioate and O-methyl-O-hydrogen S-(N-methylcarbomymethyl). A study of the fate of carbofuran and its metabolites, on strawberries was carried out by Archer *et al.* (1977).

Residue levels of carbofuran and its metabolites never exceeded the tolerance level of 0.5 ppm on the berries., nor did the carbamate fraction exceed 0.2 ppm, six days after the application of the insecticide. In some cases only the amounts of metabolites increased until seven days after application but then decreased in amount to harvest.

Volatization of dieldrin and heptachlor over a period of three weeks in summer, after the application of these insecticides to vegetation, showed that dieldrin and heptachlor were volatilized from the target area during the first twelve hours after the application. Volatilization declined rapidly over the first seven days. These are the conclusions of a study made by Taylor *et al.* (1977). They further concluded that soil and grass analysis showed that after a period of thirty days 11% of the applied dieldrin and 4% of the applied heptachlor remained in the target area; 6% of the dieldrin and 2% of heptachlor remained after fifteen weeks.

Turner *et al.* (1977) made a study on photodieldrin formation and they showed that within one day after the application of dieldrin on an orchard grass, photodieldrin residues were present. The residues accumulated to a maximum concentration of 51 ppm, five days after the application of insecticide, then slowly declined to 9 ppm. DIeldrin residues declined more rapidly and photodieldrin comprised one third to one half of the total residues after the first twenty three days.

Studies on the metabolism of isoaxathion in bean, cabbage and Chinese cabbage, using 14-carbon labelled compounds were made by Ando *et al.* (1975). Results of the studies show that the insecticide readily

penetrates into plant tissues and is hydrolyzed to produce 3-hydroxy-5-phenylisoxazole, which was then rapidly converted to water soluble compounds.

Mihara and Miyamoto (1974) investigated the metabolism of salithion, a broadspectrum organophosphorus insecticide, on bean and rice leaf surfaces. They observed that when 14-C salithion applied to bean and rice leaf surfaces vaporizes 90% and the remaining 10% is absorbed and translocated in the plants. In plants the radiocarbon insecticide is degraded by cleavage of the cyclic phosphorus ester group or undergoes demethylation to give saligenium followed by conjugation with glucose.

The translocation of C^{14}-dieldrin was studied by Phillips *et al.* (1978) using autoradiographic and scintillation techniques. They treated cotton plants with dieldrin emulsion and then kept the plants up to 40 days under controlled heat and light conditions. The results showed that the dieldrin moved laterally along the upper surface of the leaf (to which it was applied) and that 10 - 20% moved to the lower surface of the leaf.

The toxicity and persistence of cypermethrin, fenvalerate, permethrin, NRDC 161, and Shell WL 41706 was investigated against mosquitoes (*Anopheles stephensi*) and Tsetse flies *(Glossina austeni)* by Barlow, *et al.* (1977). The tests included the topical application of the insecticide, photodecomposition, sorption and decomposition on dried soils, volatility, persistence of deposits on plywood, and persistence on ivy leaves.

The results of the topical toxicity showed that NRDC 161 was the

most potent insecticide, generally all the pyrethroids were more highly toxic than the standard insecticide, dieldrin. The results of photo-decomposition shows that a comparison of permethrin and cypermethrin indicates that the presence of an alpha cyno group in the alcohol moiety reduces the stability, while the phenothrin-permethrin pair illustrates the known stabilizing effects of chlorine replacing the methyl groups in the acid portion of the molecule. Under these experimental conditions, permethrin proved to be highly resistant to light. The results on the soil study show that all the pyrethroids retained their residual toxicity due to low volatility, light stability and stability on some dried soils. The results on the persistence on plywood panels at 25°C - 26°C and 50 - 55% RH under complete darkness show that all the pyrethroids persist for 16 weeks. The persistence of permethrin and cypermethrin on Ivy leaves was detectable even after six weeks.

Hadaway *et al.* (1977), evaluated the toxicity and persistence of dieldrin, endosulfan, permethrin, and NRDC 161 against Tsetse flies (Glossina sp.). Toxicities of the insecticides were evaluated by bioassay techniques using topical application and also by contact action of the residues on Ivy leaves, which were stored at a temperature of 25°C - 26°C with petioles dipped in water for the required period of time. The results show that permethrin and NRDC 161 were more toxic than dieldrin and endosulfan when applied topically to the test insect. No loss of permethrin was recorded during the period of 29 days on the treated leaves and also no loss could be detected by internal movement of the insecticides into the leaves.

Gaughan and Casida (1978) studied the degradation of *trans* and *cis* permethrin on Cotton and Bean plants. The studies were carried

out in the field as well as under a controlled environment. The residual analysis on Cotton leaves showed that about 30% of the radiocarbon was lost within one week. The loss of radioactivity in subsequent periods was independent of isomers. *Trans* permethrin was lost more rapidly than *cis* permethrin. The treated leaves retained the radiocarbon activity completely up to three weeks. On injection into the stem of Bean plants, the permethrin isomers underwent little or no movement.

Toxicity and persistence of permethrin was evaluated against *Blatella germanica, Periplaneta americana,* and *Blatella orientalis* by Carter and Chadwick (1978). Residual toxicity and persistance of permethrin was evaluated by applying the insecticide on plywood and then storing at a temperature of 27^{o}C and 50% RH in racks under complete darkness. The results show that the initial toxicity is lost at the weekly rate of 1.9 mg^{-2}. An initial deposit of 108 mg^{-2} gave 50% kill after one year.

A series of experiments under field conditions during the summer was performed to evaluate the rate of loss of lindane, parathion, DDT, Toxaphene, aldrin, chlordane, and dieldrin on APple, Peaches, Red clover, Soya Bean, Sweet Clover, and Alfalfa (Decker *et al.*, 1950). The results showed that on apples and peaches parathion and lindane were the least persistent (21 days), followed by aldrin, chlordane, dieldrin, toxaphene and DDT. The effect of heavy rain (2.33 inches) on the residues of these insecticides gave a greater loss following rain, than when there was no rain. Toxaphene and chlordane were more resistant to heavy rains.

Laboratory and field trials were carried out to evaluate the

effectiveness and persistence of permethrin, pydrin, Shell WL 41706, methomyl with chlordimeform included for comparative purposes against *Trichoplusia ni* (Cabbage looper). The results of laboratory studies showed that three of the four pyrethroids were moretoxic than methomyl, and all four were more highly toxic than chlordimeform to 4th. instar cabbage looper. The results of post treatment temperature showed that all the four pyrethroids became increasingly toxic with decreasing temperature, the effect being most obvious with permethrin. Residue analysis showed that all the pyrethroids were more persistent than methomyl on celery with detectable residues still present 21 days after the treatment (Harris *et al.*, 1978).

Tauthongand Watters (1978), evaluated the efficacy and persistence of malathion, iodofenphos, and bromophos. Insects used for bioassay technique were confused flour beetle *(Tribolium confusum)*, Red Flour Beetle *(Orzaeaphilis surimensis)*, merchant grain beetle *(O. mercator)*, and rusty grain beetle *(Cryptolestes ferungineus)*. The insecticides were applied to plywood panels which were stored at $25^{o}C \pm 2^{o}C$ with 35 - 60% RH until required for bioassay. The results showed that all the insecticides when applied at a dose of 2.5 g/m^2 provided complete mortality of the five species for 52 weeks at a 24 hr. exposure. Malation proved to be more toxic than all the other insecticides against all the test insects except *C. ferugineus*. Persistence and speed of action of the three insecticides were directly related to the applied dosages; within the limit of exposure time and age of deposit, speed of the action can be accelerated by an increase in dosage.

2.0 MATERIALS AND METHODS

2.1 Locust Culture

The desert locust, *Schistocerca gregaria* (Forsk) was used as the bioassay organism for the studies. The main stock of desert locusts was supplied by the Centre for Overseas Pest Research, London.

The rearing procedure followed that described by Hunter-Jones (1966). Galvanized cages measuring 55 x 35 x 35 cms. were used for rearing the culture at a temperature of 26 - 30°C, with a relative humidity of 40 - 50%. Using a 40 watt bulb in each cage, enough light was provided and a photoperiod of twelve hours was maintained by an automatic electric switch.

A post treatment room with a temperature of 28 - 30°C and a RH of 40 - 50% was also utilised.

Fresh green grass, supplemented by wheat bran, was used as food for the locusts. During the severe winter and dry summer of 1976, fresh grass was short in supply so greenhouse grown brassica plants and wheat seedlings were provided.

Rearing cages were cleaned regularly by removing the faeces and dry grass. Aluminium egg tubes packed with sand were changed at regular intervals. Sterilized sand, sieved to remove particles larger than 1/10th of an inch, was used for filling the egg tubes. Before filling the tubes, the sand was moistened to provide 15% water by volume. When the egg tubes were full of 2 - 3 egg pods, they were removed from the cages,

covered with foil tops to prevent excessive evaporation and kept in an incubator at a temperature of 32°C. These egg tubes were left undisturbed for 12 - 14 days in the incubator until a day or two before the hatching was due to commence. After the eggs were hatched the tubes were removed from the incubator and the hatchlings were transferred into a clean cage. Small cages measuring 15 x 15 x 15 cms. made up of galvanised mesh were used for separating the experimental insects from the main stock.

2.2 Insecticides

Lindane, fenitrothion, and permethrin, representing three major groups of insecticide, namely organochloride, organophosphate and pyrethroid, were used for the studies. Technical grades of these insecticides were obtained from the Centre for Overseas Pest Research, London and Imperial Chemical Industries, Ltd., Jealotts Hill, Berkshire.

The insecticides and all stock solutions were kept in a refrigerator except when actually in use.

2.3 Topical application testing of insecticides

Only hoppers that had moulted to the fourth instar in the previous three or four days were used. These fourth instar hoppers were used for the reasons outlined in section 2.4; the restricted age range was used because it is known that the age of an individual within an instar can affect its susceptibility to insecticides. The site of application was also restricted because it is recognised that susceptibility can be effected by this - so the most convenient site was chosen, namely the

first and second ventral abdominal sternites.

The insecticide was made up into a 2% wt/vol solution of lindane and fenitrothion, and a 5% wt/vol solution of permethrin using KAB as the solvent. It has been shown by MacCuaig (1958) that KAB is non toxic to locusts. Waxolene red dye was added to the solution to facilitate location.

The applicator used was a graduated micro-capillary tube as described by MacCuaig and Watts (1968), in which the total volume contained was one microlitre, although using the scale the dose could be measured to within 0.01 microlitres.

The actual dose applied was calculated in terms of microgrammes of active ingredients per body weight of locust. The sex of the dosed hopper was noted, but at this age there was no significant difference in weight between the males and females.

The hoppers were treated individually in batches of ten per treatment, with usually four replicates per treatment and one untreated control group. Mortality was assessed 24 hours after treatment, the criterion of death was taken to be when the hoppers were completely immobile. No recovery from such apparently dead insects occurred, although it was expected especially from permethrin (Pojananuwong, 1976). The rather strict criterion of death within 24 hours was used largely because of convenience, but also because under field conditions relatively rapid mortality is desirable.

The experimental insects were removed from the routine culture about one hour before treatment and, immediately after treatment, each group of ten hoppers was placed in small perforated galvanized metal cages, given fresh green food and kept in the post treatment room.

2.4 Stomach Toxicity Testing

The initial tests were made using grass blades, 1½ cms. in length, which were fed to the locusts within five minutes of applying the insecticide dosage by graduated microcapillary tube. However, bearing in mind that the main object of the work involved evaluating persistence, an alternative durable substrate was necessary, because excised plant material would soon become unpalatable. So an inert material, soft tissue paper, moistened with 0.125 molar solution of sucrose, was used to investigate the effect of physical factors such as temperature, and light. Whilst living whole plants were used for tests to indicate whether any chemical degradation due to plant tissue occurred.

The same age of insect was used as for the topical application and similarly the dose was applied as before with a graduated microcapillary tube on µg/g dosage level. When persistence tests were carried out, however, a considerable time lapsed between dosing the substrate and subsequently feeding it to the locusts, so it was not possible to apply the insecticide according to body weight. Therefore, a mean weight of 0.4 g was taken as a standard. This appeared to be justified, for in this restricted age group, there is not much variation, as a test using 100 locusts (4th. instar) gave a standard error of 0.02 around a mean of 0.04 g. As described earlier, mortality was assessed

again after 24 hrs, and four replicates each of ten insects were used per treatment plus one batch of ten untreated control insects. So the pre-starved locusts were each presented with a piece of tissue paper and as soon as this was eaten the hoppers were placed in small perforated metal cages, given fresh green food and kept in the treatment room.

2.5 Effects of temperature on persistence using inert substrate

Soft tissue paper measuring 20 x 20 cms. was fastened to a glass plate with self-adhesive tape and was marked into 2 x 1 cm. rectangles. This provided sufficient treatments, adequately replicated, to allow the experiment to continue for the required number of days to enable an initial bioassay and others to follow at intervals. The required dose of insecticide was then applied to each 2 x 1 cm. area on the tissue paper and the large sheet of tissue paper was then placed in an incubator at the required temperature for the required period of time. The incubator itself varied by less than $\pm 1^{\circ}C$. The incubator interior was kept dark and the humidity was 40 - 50%.

When required for use, the tissue paper was removed from the incubator and cut into the small treated areas. Immediately before giving to the locusts, each 2 x 1 cm. piece was moistened by adding two drops of 0.125 molar solution of sucrose. A single treated sample was put into a 9cm. diameter plastic petri dish with a single fourth instar hopper.

When the whole of the tissue paper had been eaten, a period of 24 hours was allowed to elapse and then mortality was noted. All this took place in the post treatment room. Again 10 fourth instar

hoppers were used per treatment, with four replicates for each. A single batch of ten control hoppers was used & each was given a piece of 2 x 1 cm. tissue paper treated with a comparable amount of KAB, waxolene red and sucrose solution.

Abbots (1925) formula was used for the correction of mortality in the control.

2.6 Effects of light on persistence using inert substrate

The same kind of soft tissue paper was used as in 2.5, marked into rectangles as before and each rectangle treated with the appropriate amount of insecticide as described in the previous section. After treatment the sheets of tissue paper were exposed under "North Light/ Colour Matching" fluorescent tubes housed in a specially made frame on top of a horizontal incubator. The incubator itself measured 1 m x 1.5 m and the lid was clear plastic. The wavelength of the light from the fluoresence tubes was 300 - 800 nm (Fig. 1). A photoperiod of 16 hours daylight and 8 hours darkness was chosen.

The intensity of this light was 2,415.6 lux. The temperature of the inside of the incubator was set at 5°C (± 1°C) and was maintained at this level by the cooling unit of the incubator.

2.7 Persistence of insecticides on growing plants

Three different kinds of plants were used, namely wheat (*Triticum* sp.), Brussels sprouts (*Brassica olearacea gemmifera*), and privet (*Ligustrum*

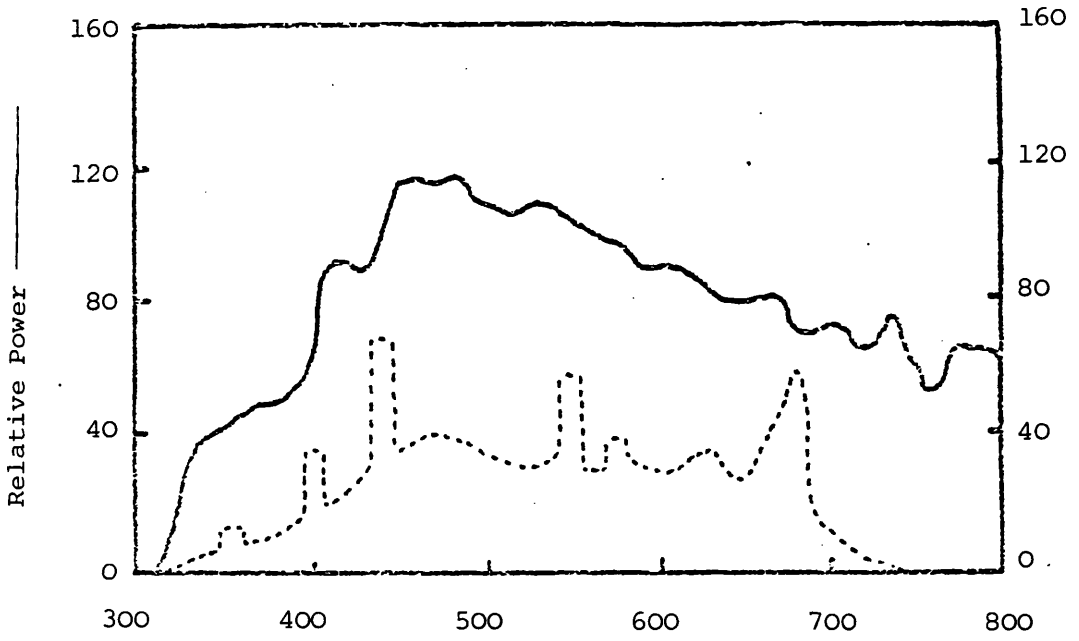

Figure. 1. Spectral Power Distribution curves for sunlight (Natural Daylight) continuous line, and Northlight Colour Matching Fluorescent lamp dashed line, ... after Henderson and Marsden. 1972

ovalifolium). Wheat and Brussels sprouts were grown in 5 cms. square plastic vacpots and were kept in a greenhouse for 14 days until the plants had developed two true leaves. The privet cuttings (1 - 1½ cms.) were taken from the parent plants and the cut ends were dipped in powder root hormone (No. 3). Excess hormone powder was removed by shaking and the cuttings struck in 5 cms. square vacpots filled with Levington Universal compost.

These were assembled in seed trays and placed under a mist propagation unit. When they had developed 2 - 4 leaves, the roots were well developed and the plants were considered ready for use.

When plants were required for experiments they were taken from the greenouse to the laboratory and 2 - 4 leaves of each plant were treated with insecticides using a microcapillary tube as described before. Treated areas were marked with a waterproof ink. After the insecticidal treatment the plants were transferred to the incubator for the required time. Their arrangement in the incubator was randomised.

Prior to giving treated plant material to the locusts in petri dishes, as described earlier, it was necessary to standardise the amount of substrate offered. This was achieved with Brussels sprouts and privet by removing 1 cm. diameter disc,s around the marked treated areas, by using a cork borer. With the thin blade of wheat a 2 cm. strip gave a piece of similar weight. Mortality was assessed after 24 hours and the number of insects, replications and post treatment procedures, were the same as described for previous experiments (2.4 and 2.5).

2.8 Effects of simulated rain conditions on the persistence of insecticides on growing plants

The same types of plants were used as described in the previous experiment (2.7) and the growing methods were similar. Water was obtained from a small reservoir that was filled by rain water from the greenhouse roofs. A water pump was connected through a hose pipe to a rosehead of a watering can. By this method water was pumped at the rate of 5 litres^{-1} hour or ½"$^{-1}$ hour. Two treatments were given, one of three hours and the other of six hours duration.

The measurement of water droplet size was carried out and Vmd was 3.2 mm and nmd was 6.2mm (Table 16 and Fig. 26). Water droplets were collected on a grease matrix and were covered quickly with liquid paraffin to prevent evaporation reducing their size. This technique has been described by Matthews (1979), who states that a suitable matrix can be made by mixing one part of petroleum jelly with two parts of light oil or medicinal paraffin. No spread factor is required as the droplets resume their original spherical shape on the surface of the matrix.

The insecticide treated plants were kept in the incubator under North light of 2415.6 lux intensity, temperature of 5^{o} ± 1^{o}C, and a RH of 50 - 55% for the required period of time. After expiry of this period, the plants were taken out of the incubator and kept under the overhead sprinkler for a period of three or six hours for simulated rain treatments. The treated leaves were later cut into circular discs (1 cm. for privet and sprouts) or strips (2 cm. for wheat) from the marked places and offered to 4th instar pre-starved hoppers.

Mortality assessment was carried out 24 hours after the leaves were offered to the locusts. The number of locusts, replications and post-treatment procedure were as described previously under 2.4 and 2.5.

2.9 Colorimetric determination of fenitrothion

Getz and Watts (1964) described a rapid method for the determination of organophosphorus pesticides. In addition to the bioassay technique, this method was used to determine the effects of temperature on the persistence of fenitrothion. The basis of the method is the reaction between nitrobenzyl pyridine and phosphate pesticides in a slightly basic solution at 175 - 180°C. As this method is rapid and has a sensitivity of 2 p.p.m. for an organophosphorus insecticide, it has obvious practical advantages.

Acetone was refluxed with 1 g of $KMNO_4$/litre for one hour and distilled. Similarly ethyl acetate was re-distilled. Nitro-pyridine solution 2% was made in refluxed acetone at weekly intervals and cyclohexamine solution 2% was prepared daily in refluxed acetone.

As a Microsnyder column and Getz tube were not immediately available, initial determinations were made using Quickfit condenser No. C2/11 and tube NO. BC 24/CHT, later the experiments were continued with a Getz tube and Microsnyder column.

The effects of temperature on the persistence of fenitrothion were determined using the tissue paper technique described in the previous section.

The various doses of fenitrothion, dissolved in acetone, were applied to the tissue paper units and left in an incubator at the required temperature and for the required time. After the expiry of the test period, the tissue paper units were taken out of the incubator and put into a test tube with 10 ml. acetone. The whirlimixer was used for 5 - 10minutes to make sure that the insecticide was completely dissolved. Then the extracted solution was transferred to a Getz tube and by using the rotary evapomixer, the solution was evaporated to dryness at 45 - 50°C.

When completely dry, 0.2 ml. of nitropyridine and 0.2 mls. of cyclohexamine solution were put in the Getz tube and the Microsnyder column was attached. Using a clamp, the lower 2" of the tube was immersed in a pre-heated (175 - 180°C) oil bath for three minutes.

Then the tube was taken out and immersed in cold water for a few seconds. Dilution to 3 ml. was done with ethylacetate and absorbance was read in 1 cm. glass cells withinten minutes. Time lapse was kept constant for every reading. Absorbance was read at 520 nm, by using an "Eel" spectrophotometer.

2.10 Statistical Analysis:

The method used for the analysis of all the screening tests is that of Minimum Logit χ^2 as described by Ashton (1972). According to this method the equation for logistic curve is

$$P = \frac{1}{1+e^{-(\alpha+\beta x)}}$$

where P is the probability of the response, i.e. death. The transformation used is

$$l = \text{logit } P = \ln\frac{P}{1-P} = \alpha + \beta x$$

when the transformed variate l is plotted against "x" dose, the points will fall on a straight line, the slope and intercept of which gives the parameters of α and β respectively, of the original function.

The observed values can be used to fit parameters to the curve and give an estimate, therefore the transformed equation for logistic curve is

$$\hat{P} = \frac{1}{1+e^{-(\hat{\alpha}+\hat{\beta}x)}}$$

After calculating the logistic curve, the goodness of fit was tested by using the logit χ^2 as

$$\text{logit } \chi^2 = n\dot{\{} W'(1-\hat{l})^2 \text{ on } n-2 \text{ df}$$

where as n is the number of dose level used.

To calculate the lethal doses (denoted as LD_{50}, LD_{80} and LD_{90}), the following equations are used:

$$LD_{50} = -\frac{\hat{\alpha}}{\hat{\beta}}$$

$$LD_{80} = \frac{\ln 4-\hat{\alpha}}{\hat{\beta}}$$

$$LD_{90} = \frac{\ln 9-\hat{\alpha}}{\hat{\beta}}$$

For the calculation of standard errors for different lethal doses see appendix.

Confidence intervals for LD_p are given by

$$L\hat{D}_p \pm Z_\alpha \sqrt{\text{standard error of } L\hat{D}_p}$$

where Z_α is the appropriate percentage point of the normal distribution taken to give a two tailed probability of 0.05 throughout the analysis. Confidence intervals were calculated for 50%, 80% and 90% lethal doses.

The statistical analysis of the colorimetric determination was carried out through programmed calculator, i.e. Hewlett Packard 97 (Standard Pack). The programme used was "curve fitting" under which the following programme can be used to fit data to:

1. Straight line (linear regression)
2. Exponential curve
3. Logarithmic curves
4. Power Curves

It was decided to use the linear regression for the statistical analysis. The equation for linear regression is described below:

$$Y = a + bx$$

where "a" is the parameter of intercept and "b" is the parameter of slope. Whereas

$$b = \frac{\Sigma x_i y_i - \frac{\Sigma x_i - \Sigma y_i}{n}}{\Sigma x_i^2 - \frac{(\Sigma x_i)^2}{n}}$$

$$a = \frac{\Sigma y_i}{n_1} - b \frac{\Sigma x_i}{n}$$

$$r^2 = \frac{\left(x_i y_i - \frac{\Sigma x_i \Sigma y_i}{n}\right)^2}{\left(\Sigma x_i^2 - \frac{(\Sigma x_i)^2}{n}\right)\left(\Sigma y_i^2 - \frac{(\Sigma y_i)^2}{n}\right)}$$

3.0 Preliminary Insecticide Tests

3.1 Introduction

In many, if not most agricultural situations when insecticides are used against a pest, the aim is to hit the pest individuals directly with the toxic material (however formulated) and also to leave a residual deposit on the crop so that pest individuals that subsequently eat the crop, or walk over the crop, will then receive a lethal dose. That is, both direct contact action and residual contact or stomach action are involved. For this reason, therefore, both the contact and stomach action effects of the three chosen insecticides were investigated.

For topical application, the technique of applying doses according to the weight of the individual were used as described in Section 2.3. In the initial stomach testing, the most convenient substrate was to use single pieces of grass leaves, each 1½ to 2 cms. in length, as described by Busvine (1971). However, the single grass leaf technique was not suitable for experiments on persistence as the excised grass becomes desiccated and is then less palatable to the locusts. Furthermore, it was hoped to determine any differences between persistence on inert surfaces and persistence on a variety of growing plants' surfaces. So a suitable inert surface was required. Tests with standard filter paper, rice paper, biscuit wafers, and soft tissue paper were made; it was found that soft tissue paper was the most satisfactory, after it had been moistened with 0.125 molar sucrose solution. Other materials tested as additives were water, citric acid, corn oil, and grass extract but these were less satisfactory than sucrose.

The test piece of sucrose treated tissue paper was usually completely eaten within ten minutes of being offered to locusts. Even when the required dose of lindane and fenitrothion was added, the hoppers showed no aversion and the paper was still eaten in the 10 minute period. Permethrin, however, appeared to have some repellant properties and the period required for total consumption increased to 15 - 20 minutes.

Fifth instar hoppers were tested using this technique but were found to be less reliable than fourth instar hoppers. Eventually, after a number of tests the method was standardised so that nymphs, that had moulted to the fourth instar in the previous 3 to 4 days, were starved for 48 hours before the tests, then given the 3½ x 1½ cm. piece of tissue paper treated with the appropriate amount of insecticides. Mortality was assessed 24 hours after the paper was eaten.

3.2 Experiments with lindane

3.21 Topical application experiments

Hoppers of the desert locust, that had moulted to the fourth instar three/four days previously, were used and randomly divided into groups of ten individuals per group. Six dosage rates of 3, 5, 8, 12 and 14 µg/g were used. Choice of dose rates was based on the results from MacCuaig (1966) and the individual doses ascended in $\sqrt{2}$ progression. One replicate of each dose was made, with ten insects per group , and one control for each group was treated with the solvent KAB and waxoline red. The amount of solvent used was equivalent to the dose of insecticide applied. Mortality was assessed after 24 hours (see Section 2.3). The results showed that mortality increased with ascending doses of lindane, but that the LD_{50} dose was 8.4 µg/g, the LD_{80}

was 11.4 µg/g and the LD_{90} was 13.5 µg/g. The numerical values for the parameters of intercept and slope ($\hat{\alpha}$, $\hat{\beta}$) are -3.878 and 0.462 respectively. See Tables 1 and 4 and Appendix 2.

3.22 Stomach toxicity using grass as substrate

The same experimental procedure was used as in the previous section, except that the hoppers were starved of food for 48 hours before the test. Again the choice of dosage rates was based on MacCuaig (1966) and these were applied to grass instead of topically. The results for stomach toxicity showed again increasing mortality with increased dosage of poison, and the LD_{50}, LD_{80} and LD_{90} dosages were 7.9 µg/g, 10.4 µg/g, and 11.8 µg/g respectively. The parameters of intercept ($\hat{\alpha}$) and slope ($\hat{\beta}$) were -4.500 and 0.567 respectively. See tables 1 and 5 and Appendix 6.

3.23 Stomach toxicity using tissue paper as substrate

Apart from the substitution of pieces of tissue paper for grass, the experimental procedure was the same as in the previous experiment. The results of this study show that the tissue paper method required an LD_{50} of 5.2 µg/g, LD_{80} of 6.9 µg/g, and LD_{90} of 7.9 µg/g.

The values for the intercept and slope ($\hat{\alpha}$, $\hat{\beta}$) were -4.256 and 0.812 respectively. The calculations for χ^2 used in testing the fit of the estimated line are given in Appendix 11 and show a value of 1.628. The values for different standard errors and 95% CI are presented in Tables 1 and 6 and Appendix 10.

Table 1. Susceptibility to Lindane of 3-4 day old 4th. instar hoppers of the desert locust.

Methods	Parameter Estimates		Estimated Lethal Doses (µg/g) & Standard Errors						95% C.I.for
	$\alpha\hat{}$	$\beta\hat{}$	LD_{50}	S.E.	LD_{80}	S.E.	LD_{90}	S.E.	LD_{50}
Contact poisoning	-3.878	0.462	8.391	0.749	11.394	1.708	13.149	2.380	6.69,10.09
Stomach poisoning with grass	-4.500	0.567	7.930	0.610	10.380	0.880	11.810	1.37	6.40, 9.46
Stomach poisoning with tissue paper	-4.256	0.812	5.240	0.310	6.940	0.400	7.950	0.60	4.15,6.33

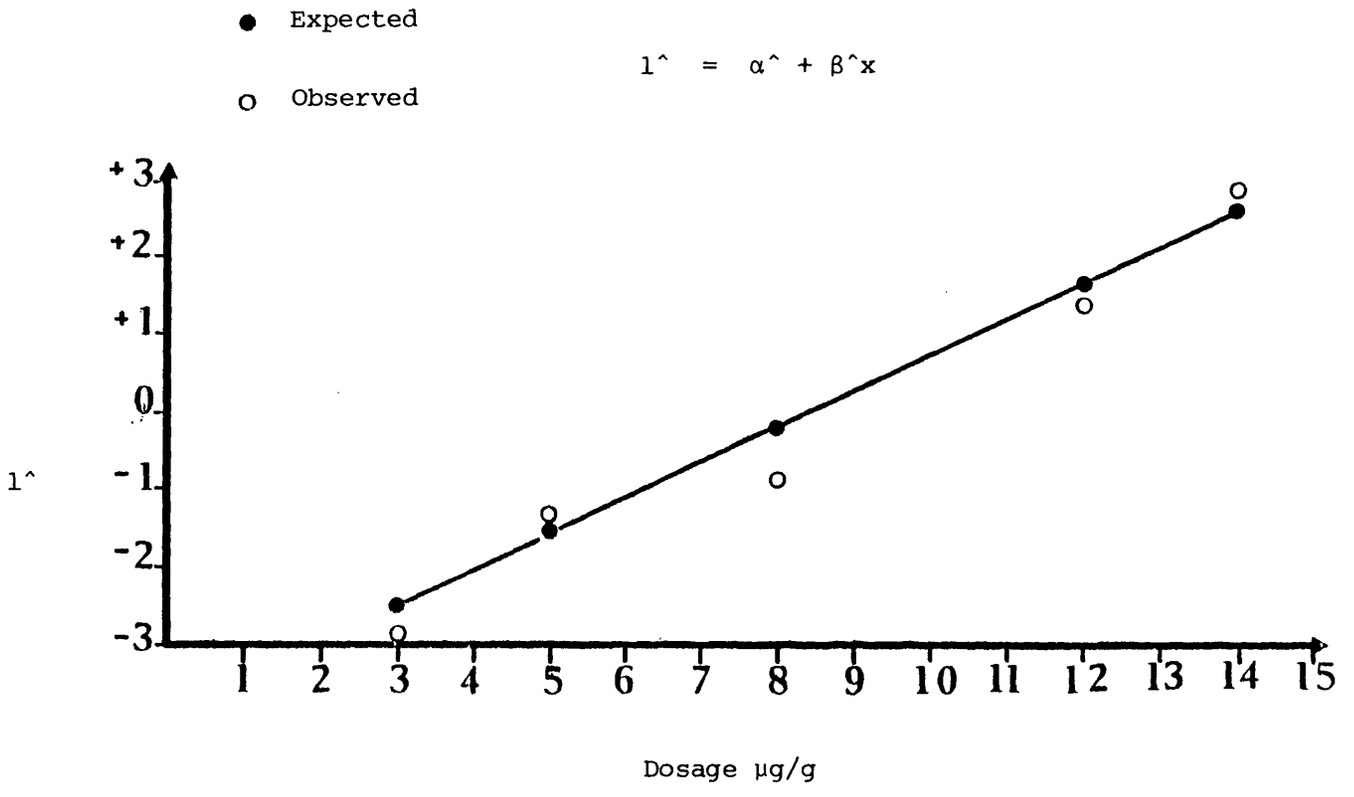

Figure 2. Fitted line for lindane contact poisoning

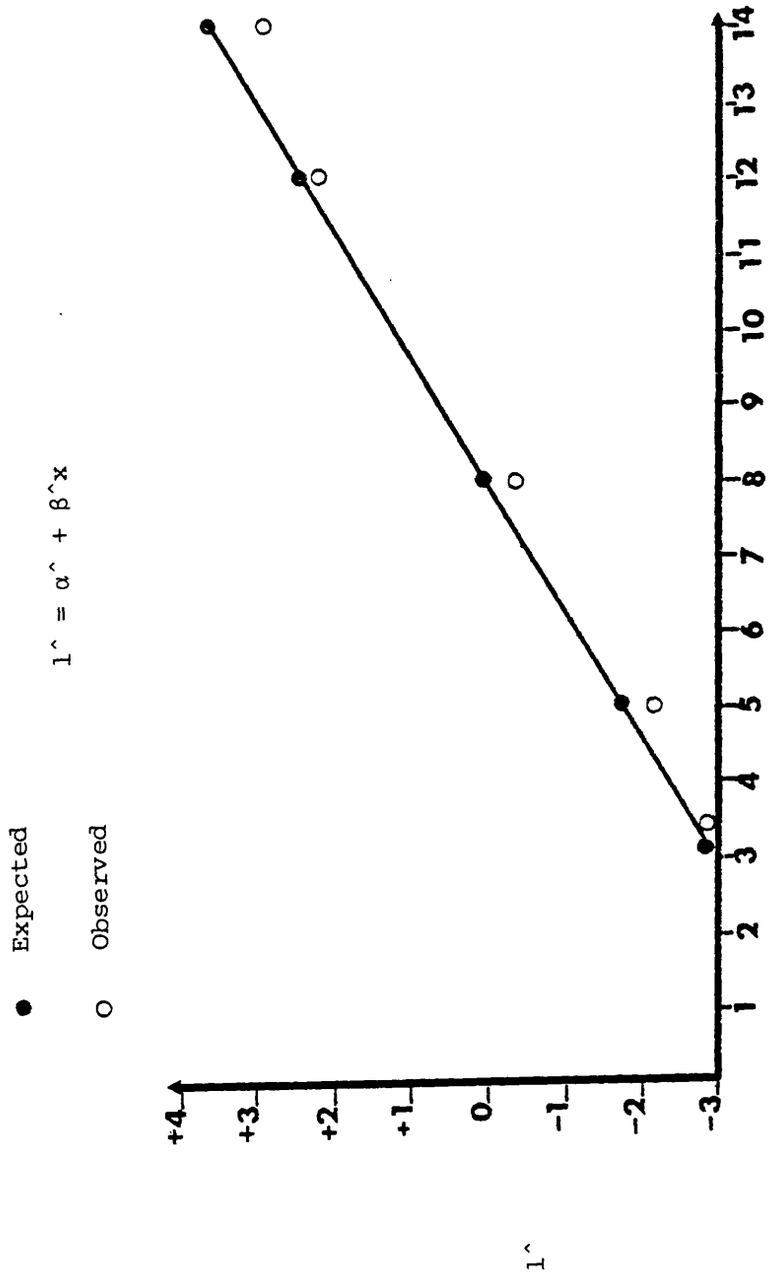

Figure 3. Fitted line for lindane stomach poisoning (Grass)

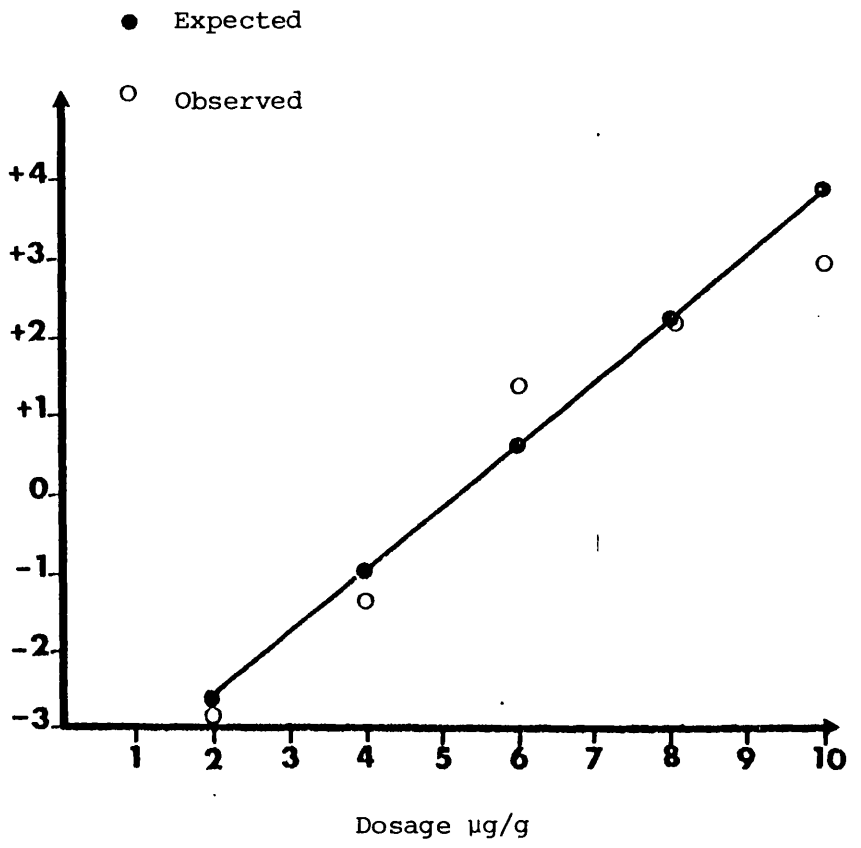

Figure 4. Fitted line for lindane stomach poisoning (tissue paper)

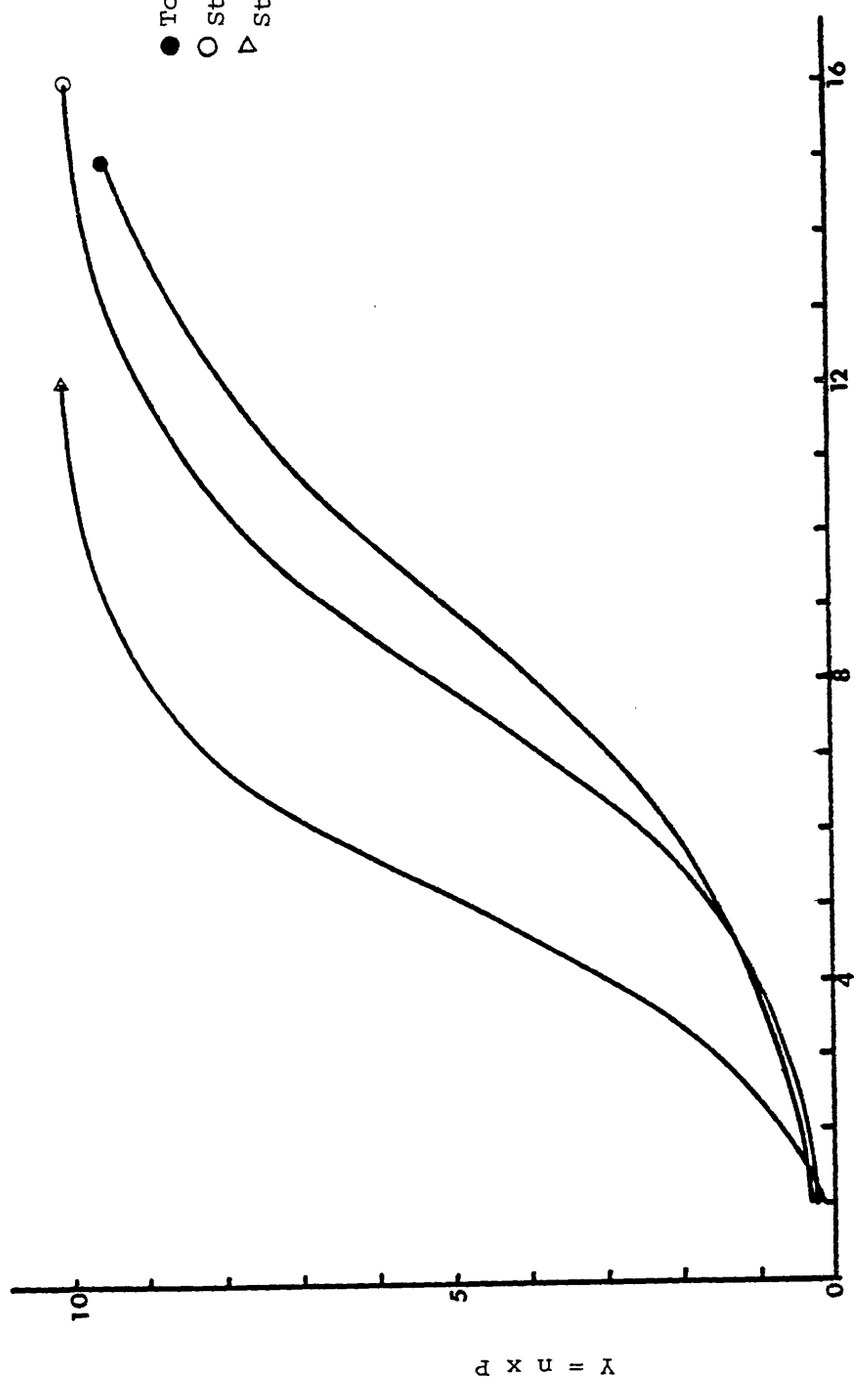

Figure 5. Logistic curves for lindane poisoning

3.3 Experiments with fenitrothion

The same experimental procedures were used as described in the previous section for lindane.

3.31 Topical application experiments

A summary of the results obtained by the toxicity tests with fenitrothion are presented in Table 2, which shows that for contact poisoning a LD_{50} of 5.2 µg/g, LD_{80} of 7.0 µg/g and LD_{90} of 8.1 µg/g were obtained.

The numerical values for the parameter of intercept ($\alpha\hat{}$), and slope ($\beta\hat{}$) obtained were -3.829 and 0.742 respectively.

The calculations for logit χ^2 are presented in Appendix 15 and the value χ^2 for contact poisoning is 8.5. The 95% CI for LD_{50} is presented in Tables 2 and 4 which is 4.4, 5.9; for all other lethal doses see Appendix 14.

3.32 Stomach toxicity using grass as substrate

Results of this toxicity test show that LD_{50} value is 7.5 µg/g, LD_{80} is 10.9 µg/g, and LD_{90} is 12.8 µg/g. The value for the parameter of intercept ($\alpha\hat{}$) is -3.102, and for the parameter of slope ($\beta\hat{}$) is 0.143, whereas the χ^2 value is 2.5 which is not significant (Appendix 19).

The 95% CI for all the lethal doses and the values for standard errors are presented in Tables 2 and 5 and Appendix 18.

Table 2. Susceptibility to Fenitrothion of 3-4 day old 4th. instar hoppers of desert locust

Methods	Parameter Estimates		Estimated Lethal Doses (µg/g) & Standard Errors						95% CI for LD_{50}
	$\alpha\hat{}$	$\beta\hat{}$	LD_{50}	S.E.	LD_{80}	S.E.	LD_{90}	S.E.	
Contact poisoning	-3.829	0.742	5.160	0.157	7.030	0.221	8.120	0.369	4.380,5.940
Stomach poisoning with grass	-3.102	0.413	7.510	0.583	10.867	0.840	12.831	1.421	6.000,8.990
Stomach poisoning with tissue paper	-2.650	0.370	7.160	0.370	10.910	1.150	13.100	2.177	5.970,8.340

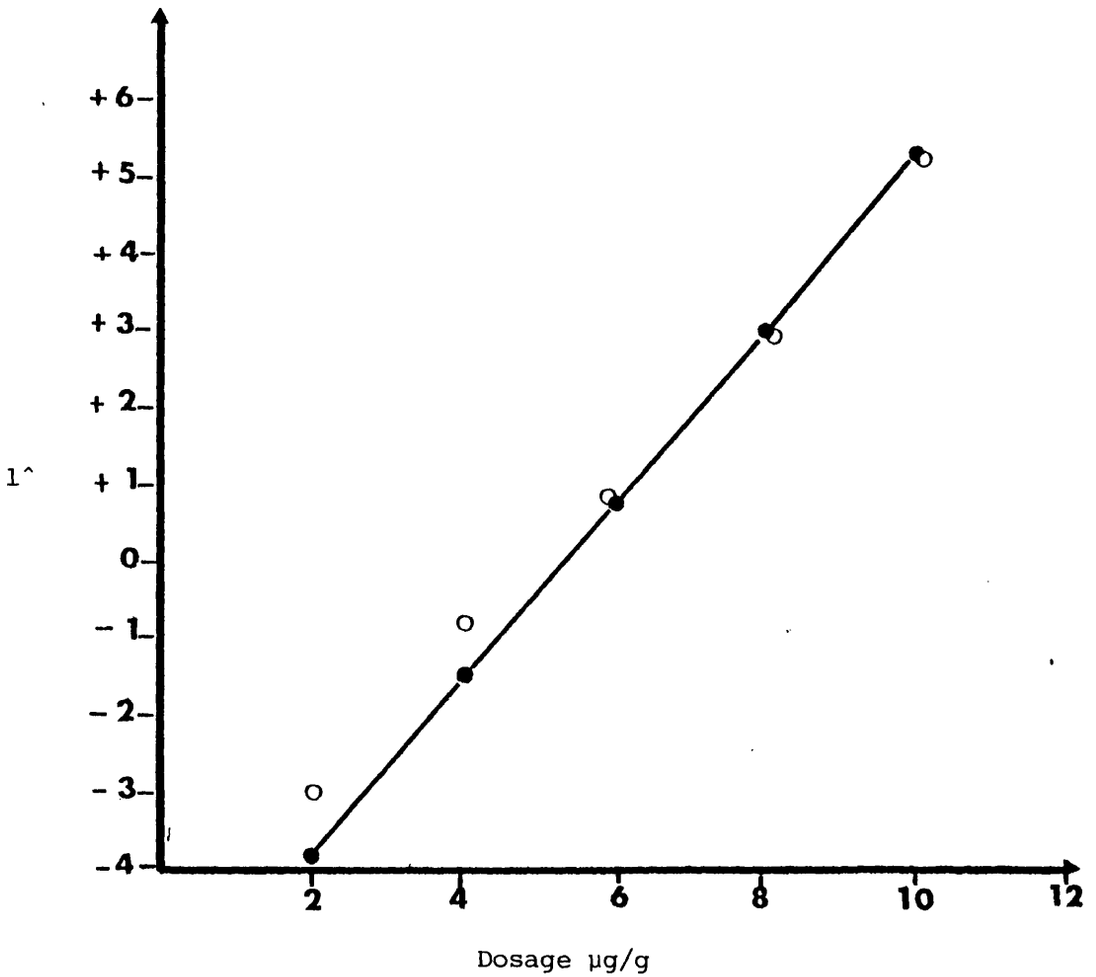

Figure 6. Fitted line for fenitrothion contact poisoning

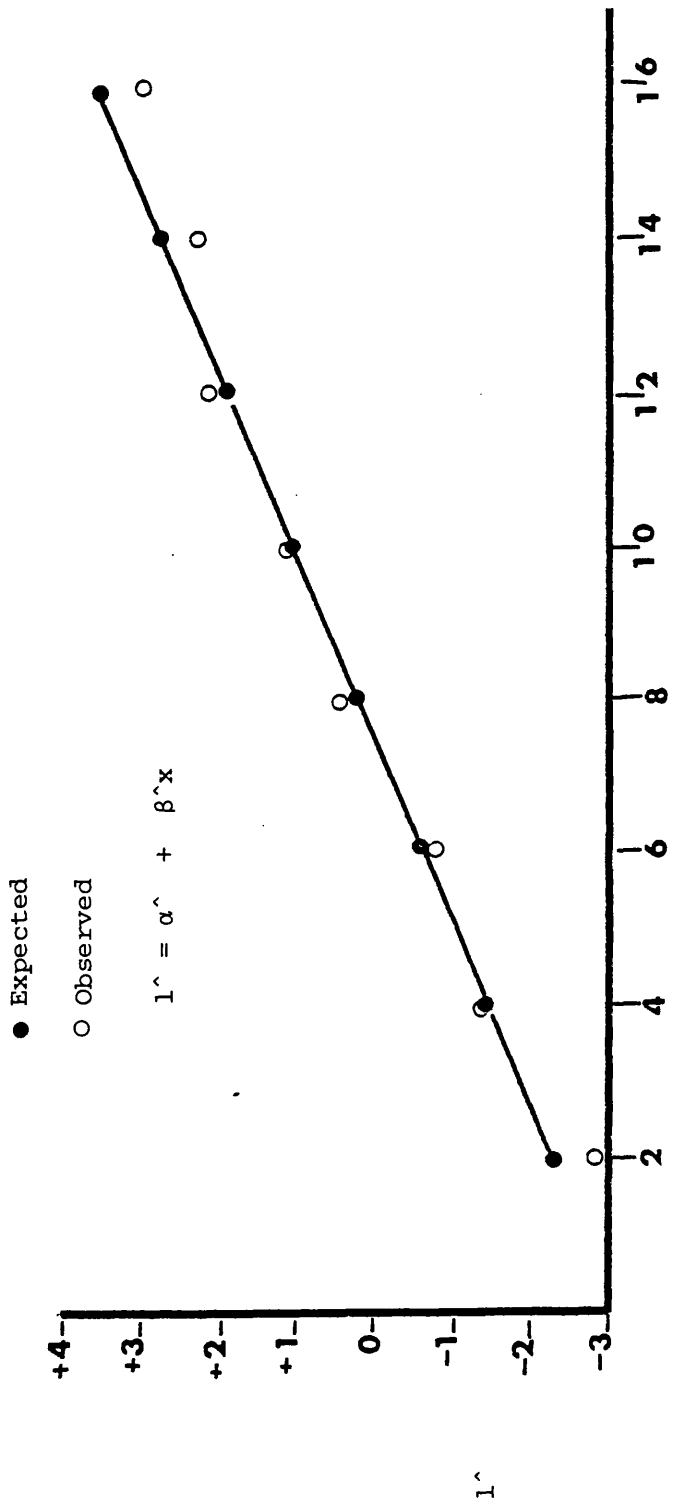

Figure 7. Fitted line for fenitrothion stomach poisoning (grass)

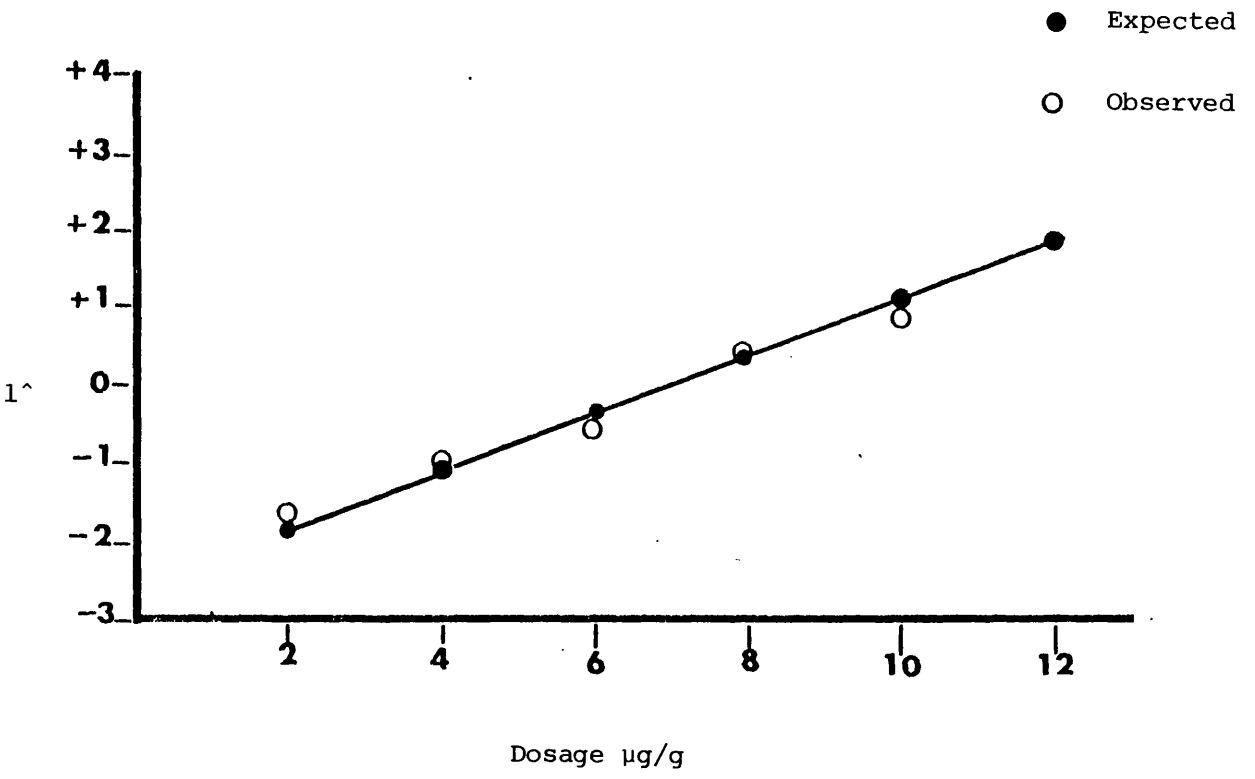

Figure 8. Fitted line for fenitrothion stomach poisoning (tissue paper)

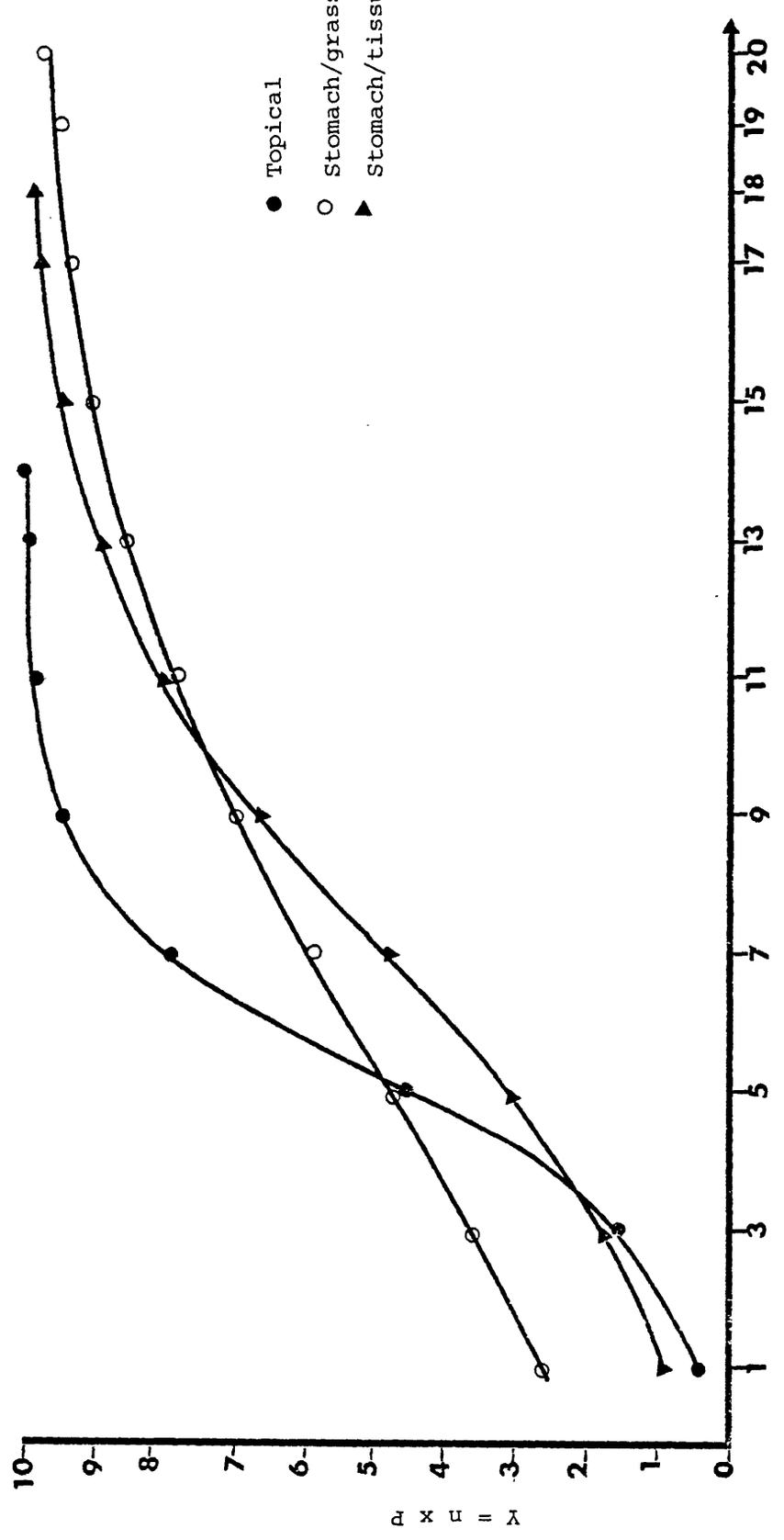

Figure 9. Logistic curves for fenitrothion

3.33 Stomach toxicity using tissue paper as substrate

The results show LD_{50} of 7.2 µg/g, LD_{80} of 10.9 µg/g and LD_{90} of 13.1 µg/g.

The values of intercept ($\alpha\hat{}$) and slope ($\beta\hat{}$) are -2.650 and 0.370 respectively. The standard errors and 95% CI values are presented in Tables 3 and 6 and Appendix 22. The value of χ^2 is 2.2, which is not significant (Appendix 23).

3.4 Experiments with permethrin

3.41 Topical application experiment

For experimental procedures see Section 3.2. The summarized results obtained by toxicity tests with permethrin are presented in Table 3. From this table it can be seen that for contact poisoning, the LD_{50} is 11.6 µg/g, LD_{80} is 16.0 µg/g, and LD_{90} is 18.7 µg/g.

The values for standard errors and 95% CI are presented in Tables 3 and 4 and Appendix 26. The value for logit χ^2 = 2.426, which is not significant (Appendix 27).

3.42 Stomach toxicity using grass as substrate

The results show that LD_{50} is 27.1 µg/g, LD_{80} is 32.1 µg/g and LD_{90} is 34.9 µg/g. The values for 95% CI, and standard errors are presented in Tables 3 and 5 and Appendix 30.

The numerical values for the parameter of intercept, and slope ($\alpha\hat{}$, $\beta\hat{}$) are -7.59 and 0.28 respectively. The logit χ^2 value is 0.767, which is not significant (Appendix 31).

3.43 Stomach toxicity using tissue paper as substrate

The LD_{50} obtained is 36.8 µg/g, LD_{80} is 43.8 µg/g, and LD_{90} is 47.9 µg/g. Tables 3 and 6 and Appendix 34 show the 95% CI values and the values for standard errors. The χ^2 value is presented in Appendix 35, which is not significant.

The parameters of intercept and slope ($\alpha\hat{}$, $\beta\hat{}$) gave the values of -7.28 and 0.198 respectively (Appendix 34).

3.5 Summary and Discussion

The results from the three insecticides tested show that in terms of comparative toxicity, fenitrothion was the most effective insecticide for use against locusts; lindane was intermediate; whilst permethrin was the least effective. Similarly, when grass was used as a substrate for stomach toxicity, fenitrothion was the most toxic; lindane next and permethrin was the least effective. When tissue paper was used as a substrate for stomach toxicity, however, lindane was the most effective, followed next by fenitrothion and finally again by permethrin.

Comparing the methods of application of the insecticide within a single chemical, it was clear that for both fenitrothion and permethrin the contact application was the most effective in terms of µg/g wt. of locust, than was the stomach poison method. However, for fenitrothion

Table 3. Susceptibility to Permethrin of 3-4 day old 4th. instar hoppers of the desert locust

Methods	Parameter Estimates		Estimated Lethal Doses (µg/g) & Standard Errors						95% C.I. for LD_{50}
	$\alpha\hat{}$	$\beta\hat{}$	LD_{50}	S.E.	LD_{80}	S.E.	LD_{90}	S.E.	
Contact poisoning	-3.600	0.310	11.590	1.363	16.050	2.380	18.660	4.120	9.300,13.88
Stomach poisoning with grass	-7.590	0.280	27.100	1.240	32.060	2.390	34.950	4.200	24.920,29.280
Stomach poisoning with tissue paper	-7.287	0.198	36.800	1.300	43.800	2.810	47.900	5.560	34.560,39.030

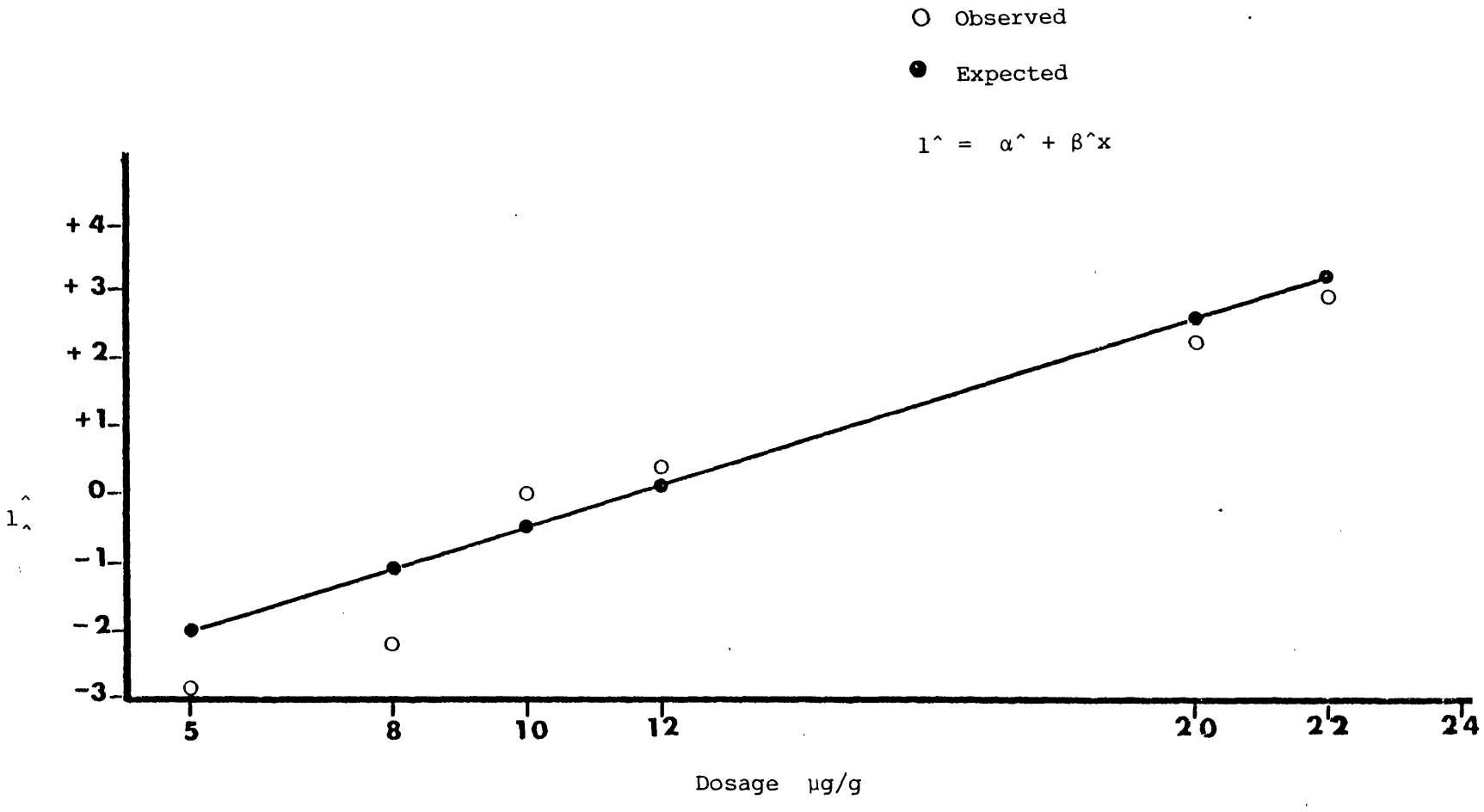

Figure 10. Fitted line for permethrin contact poisoning.

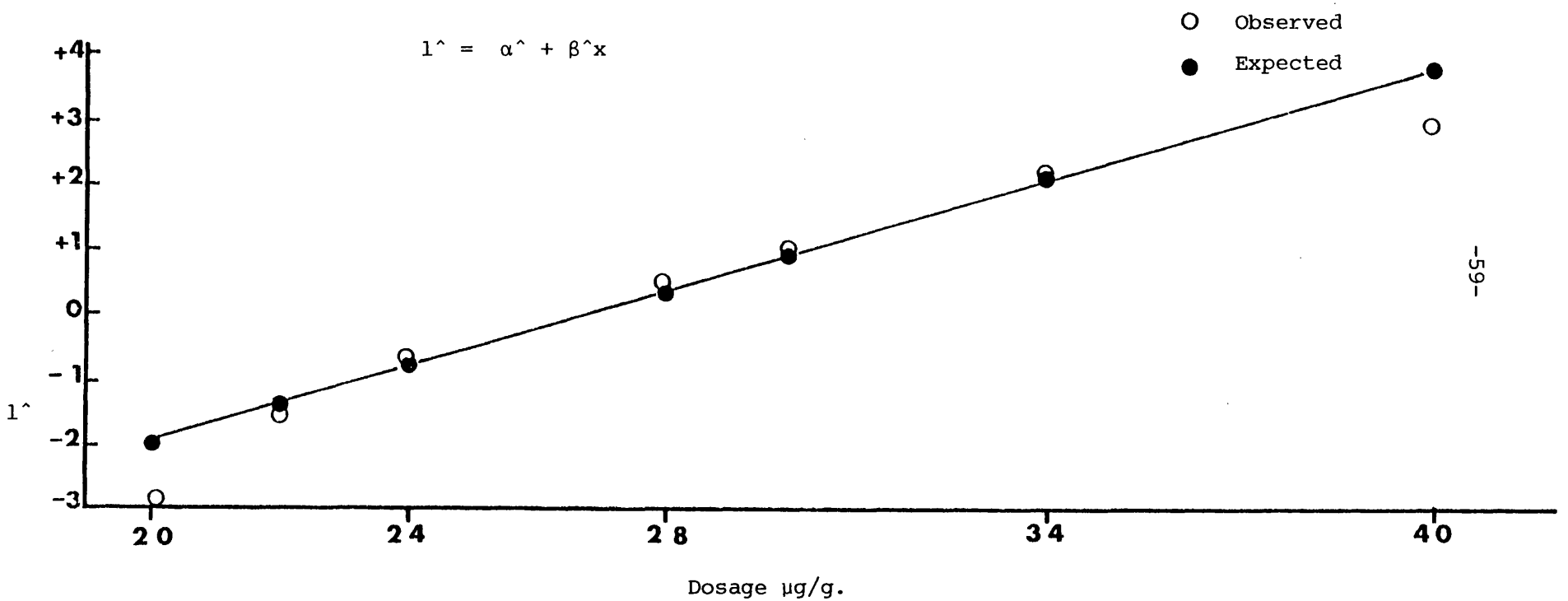

Figure 11. Fitted line for permethrin stomach poisoning (grass).

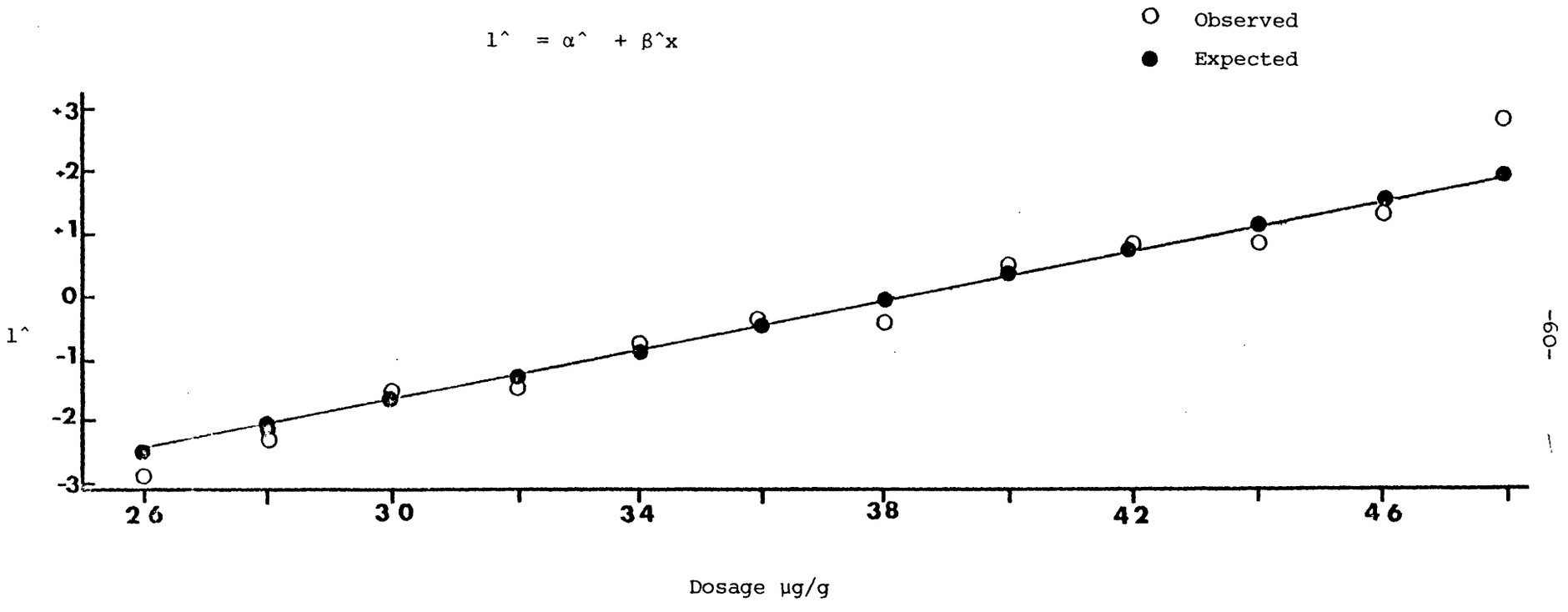

Figure 12. Fitted line for permethrin stomach poisoning (tissue paper).

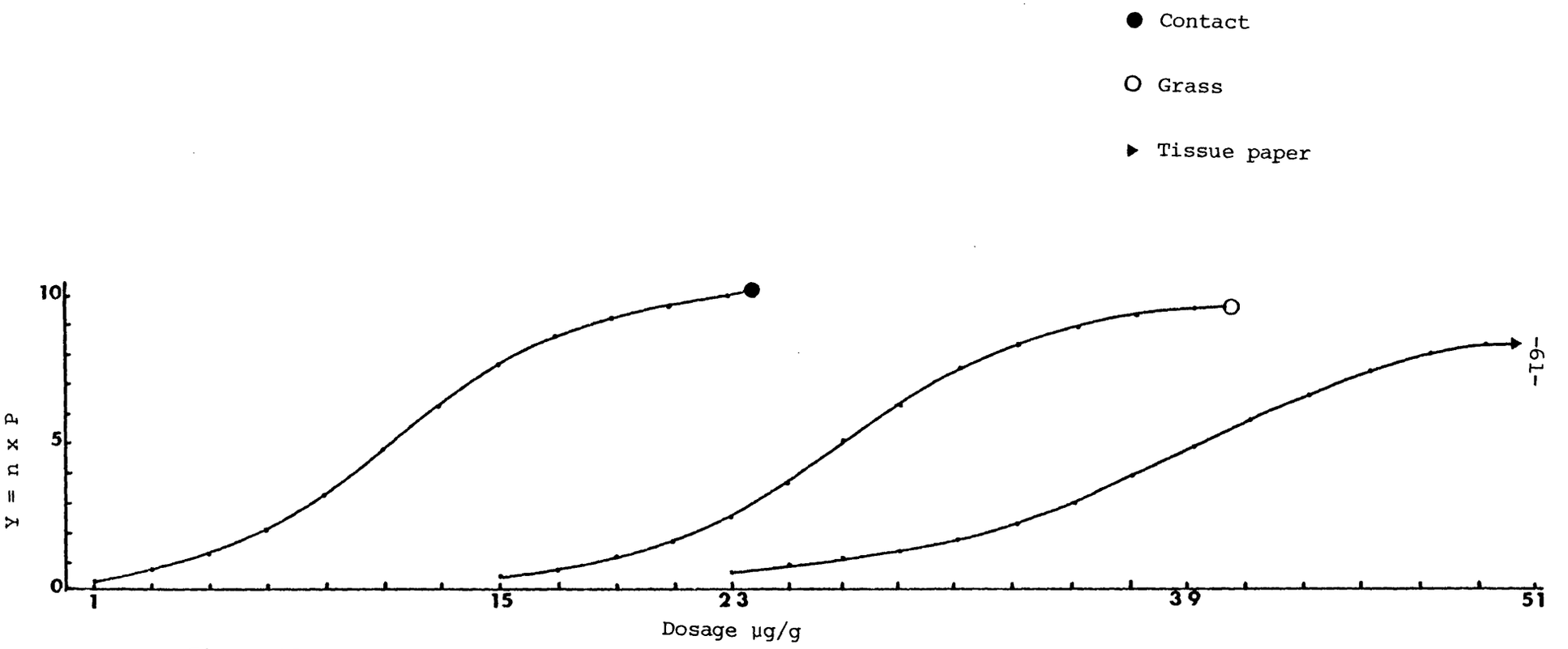

Figure 13. Logistic curve for permethrin

Table 4. Comparative statement showing the results obtained from contact poisoning with Lindane, Fenitrothion and permethrin

Contact Poisoning	Lethal Doses (µg/gm) & Confidence Intervals					
	LD_{50}	95% C.I.	LD_{80}	95% C.I.	LD_{90}	95% C.I.
Lindane	8.391	6.69,10.09	11.394	9.06,13.71	13.149	10.12,16.17
Fenitrothion	5.160	4.38, 5.94	7.030	6.10, 7.95	8.120	6.93, 9.31
Permethrin	11.590	9.30,13.88	16.050	13.02,19.07	18.660	14.68,22.63

Table 5. Comparative statement showing the results obtained from stomach poisoning with grass, using lindane, fenitrothion and permethrin.

Stomach poisoning with grass	Lethal Doses (µg/gm) and confidence intervals.					
	LD_{50}	95% CI	LD_{80}	95% CI	LD_{90}	95% CI
Lindane	7.93	6.41, 9.46	10.38	8.54, 12.21	11.81	9.51, 14.10
Fenitrothion	7.510	6.00, 8.99	10.867	9.07, 12.66	12.831	10.49, 15.16
Permethrin	27.100	24.92, 29.28	32.060	29.02, 35.09	34.950	30.93, 38.96

Table 6. Comparative statement showing the results obtained from stomach poisoning with tissue paper, using Lindane, Fenitrothion and Permethrin

Stomach poisoning with tissue paper	Lethal Doses (µg/gm) & Confidence Intervals					
	LD_{50}	95% C.I.	LD_{80}	95% C.I.	LD_{90}	95% C.I.
Lindane	5.240	4.15,6.33	6.940	5.70,8.18	7.950	6.42,9.46
Fenitrothion	7.160	5.97,8.34	10.910	8.80,13.01	13.100	10.20,15.99
Permethrin	36.800	34.56,39.03	43.800	40.51,47.08	47.900	43.27,52.52

the tissue paper substrate was more effective than was the grass blades, but in permethrin the grass was more effective than tissue paper. But with lindane both stomach methods were more effective than the contact method; of the stomach methods, the tissue paper was more effective than the grass methods.

It is clear from this work, that presenting a known dose of insecticide on the soft tissue paper to a 4th. instar locust hopper, in order to investigate stomach toxicity, is a satisfactory method, giving repeatable and meaningful results. This method was therefore adopted as standard procedure for the subsequent experiments on persistence.

4.0 Persistence on Inert Material

4.01 General Introduction

Once an insecticide has been applied in a field situation it comes under the influence of several environmental factors. Temperature is probably one of the most important, but light, in terms of solar radiation, is likely to be responsible for some degradation. Also rainfall may be expected to affect persistence possibly by simply washing the insecticide off the leaf or, perhaps by direct reaction of the insecticide with free moisture. Wind may be of some significance if the insecticide is volatile, i.e. has a low vapour pressure. In addition it could modify the degradation effect of rains by altering the angle of incidence

4.1 Effects of Temperature on Persistence

4.11 Introduction

Temperature is considered to be one of the principal factors affecting the persistence of an insecticide under natural conditions (Section 6.0). The effect would be maximised under tropical conditions, where the majority of pests occur and where temperatures are highest.

For the present section, therefore, the tissue paper method of feeding doses of insecticide was used as it was shown to be satisfactory in the previous section. Two dosage rates for each of the three insecticides were used, namely the dosage that has been shown to give the stomach LD_{50} and that shown to give the stomach LD_{90} level (Sections 3.2 to 3.5). So for each insecticide, the LD_{50} or LD_{90} dosages were placed on each of many tissue rectangles (Section 2.5). The dosed paper was then held at the required temperature for the required period

of time before being fed to the locusts and subsequent mortality noted. The temperatures used were between 5°C and 30°C.

4.12 Experiments with lindane

Tissue papers treated with the LD_{50} for stomach action, were fed to locust hoppers within five minutes of treatment. Ten hoppers per treatment and four replicates were used each time with another 10 hoppers serving as a control. When mortality was assessed after 24 hours it was seen that 55% of the locusts died, confirming the reasonable accuracy of the dosage rates. By the time the papers had been stored at 5°C for three days, however , the mortality had fallen to 2.5% and after seven days storage there was no observed mortality (Table 7 and Fig. 14). When the papers were kept at 15°C, the mortality was again 2.5% after three days, and zero mortality after seven days. The results for papers initially dosed with LD_{90} level were essentially similar in that the mortality to locusts fed the papers immediately after dosing was 95%, but when kept at 5°C the mortality after three days was 15% and zero after seven days. Similarly papers dosed with the LD_{90} level and kept at 15°C showed that the mortality had fallen to 2.5% after three days and to zero after seven days (Table 7 and Figs. 14 and 15).

4.13 Experiments with fenitrothion

The experimental procedure was similar to that as described under 4.12 except that the effect of three temperature levels, namely 5°C, 10°C and 15°C were investigated.

Time period	5°C		15°C	
	LD_{50} 5.24 µg/g	LD_{90} 7.95 µg/g	LD_{50} 5.24 µg/g	LD_{90} 7.95 µg/g
	Mortality %		Mortality %	
Nil	55	95	55	95
3 days	2.5	15	2.5	2.5
7 days	0	0	0	0

Table 7. The effects of temperature on the persistence of lindane.

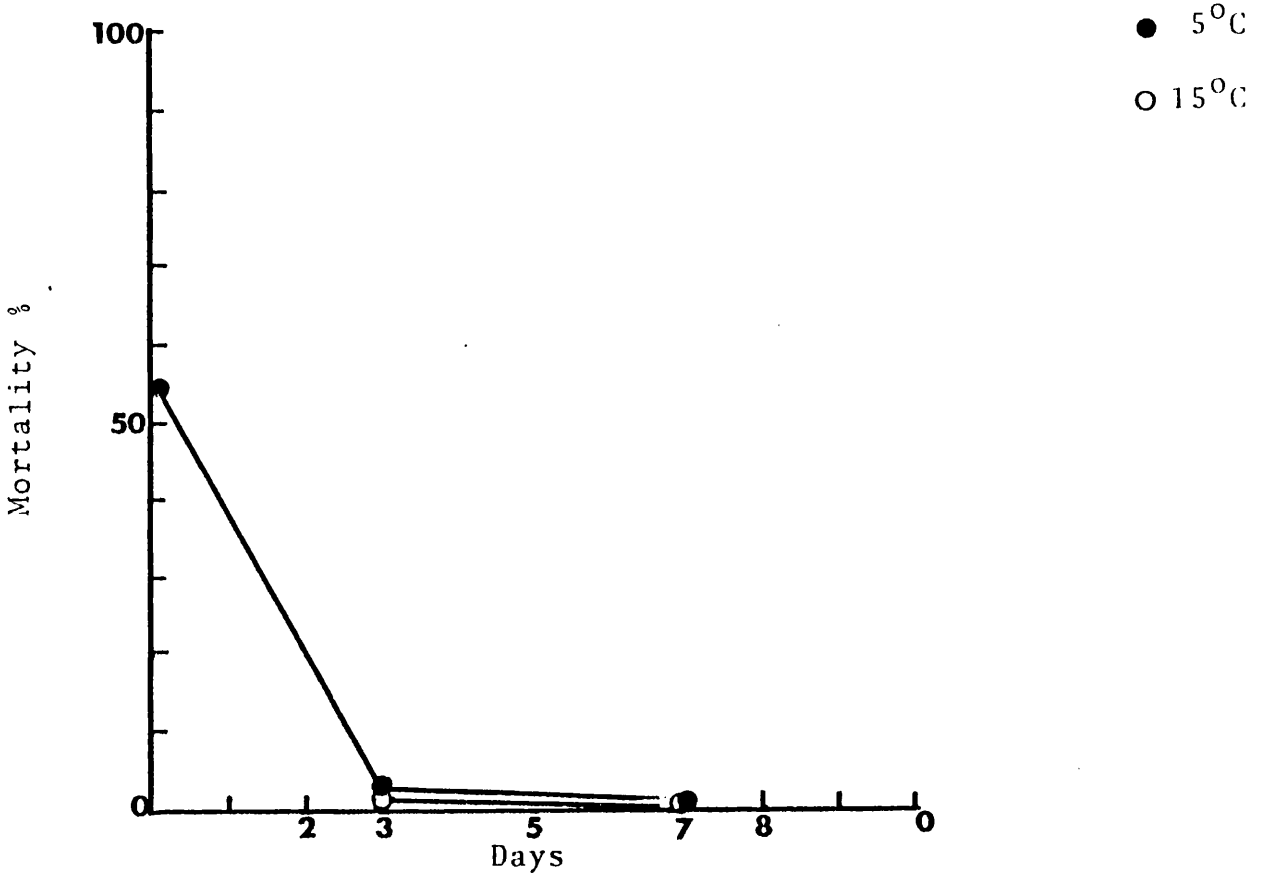

Figure 14. Effects of temperature on the persistence of lindane (LD_{50}).

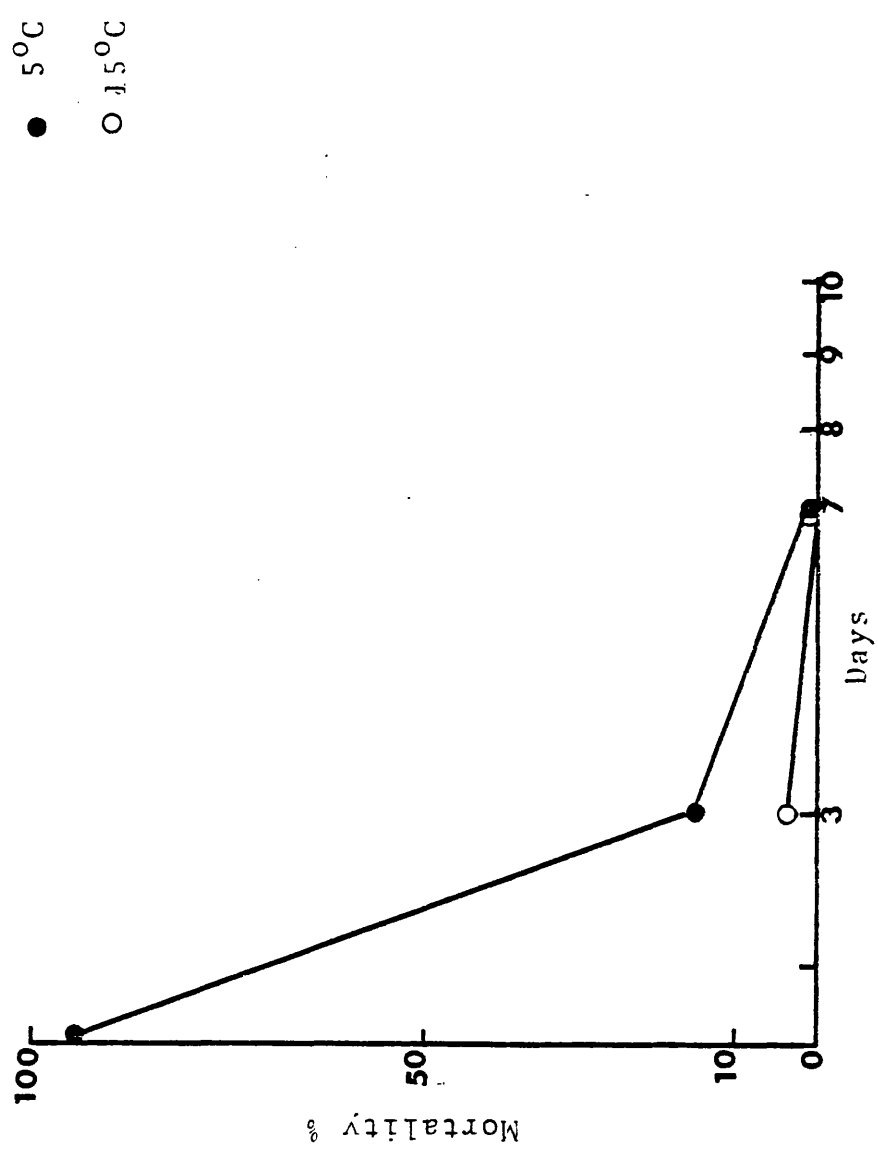

Figure 15. Effects of temperature on the persistence of lindane (LD_{90}).

The results of feeding fourth instar hoppers with tissue paper immediately after the paper had been treated with the LD_{50} dosage level showed a mortality of 60%, so confirming the results obtained previously by toxicity tests. After three days storage at 5°C the mortality assessed was 30%, which was further reduced to 8% after seven days and zero mortality after fourteen days.

The results of immediate feeding of LD_{90} level to the locusts was 97%, but when stored at 5°C for three days the mortality obtained was 93%, which decreased after a period of seven days to 50%, further storage of fourteen days showed a mortality of 8%.

The results of storage of LD_{50} at 10°C for three days showed a mortality of 32%, whereas storage of seven days and fourteen days showed a mortality of 7% and 0% respectively. The results of LD_{90} storage at 10°C for three days gave a mortality of 95%, whereas 20% and 0% mortality were recorded as a result of storage for seven days and fourteen days.

The persistence of fenitrothion at 15°C for three days storage showed a mortality of 17%, and seven days storage showed a mortality of 0%. The storage of LD_{90} at the same temperature for three days showed a mortality of 17%, and the storage of seven days gave a mortality of 0%.

All the above results are presented in Table 8 and Figures 16 and 17.

Table 8. Effects of temperature on the persistence of fenitrothion.

Time	Temperature 5°C		Temperature 10°C		Temperature 15°C	
Period	LD_{50} 7.16 µg/g	LD_{90} 13.10 µg/g	LD_{50} 7.16 µg/g	LD_{90} 13.10 µg/g	LD_{50} 7.16 µg/g	LD_{90} 13.10 µg/g
	Mortality %		Mortality %		Mortality %	
Nil	60	97	60	97	60	97
3 days	30	93	32	85	17	17
7 days	8	50	7	20	0	0
14 days	0	8	0	0	-	-

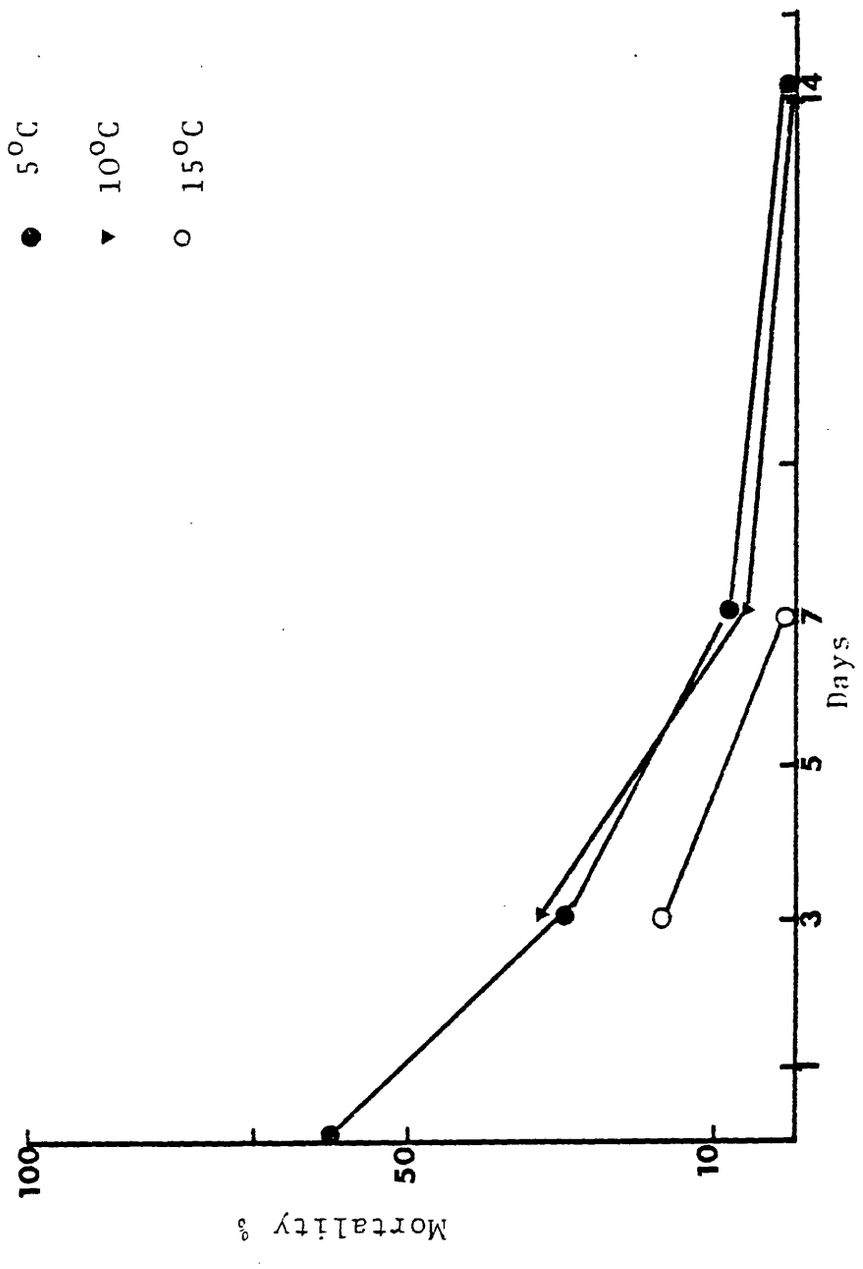

Figure 16. Effects of temperature on the persistence of fenitrothion (LD_{50})

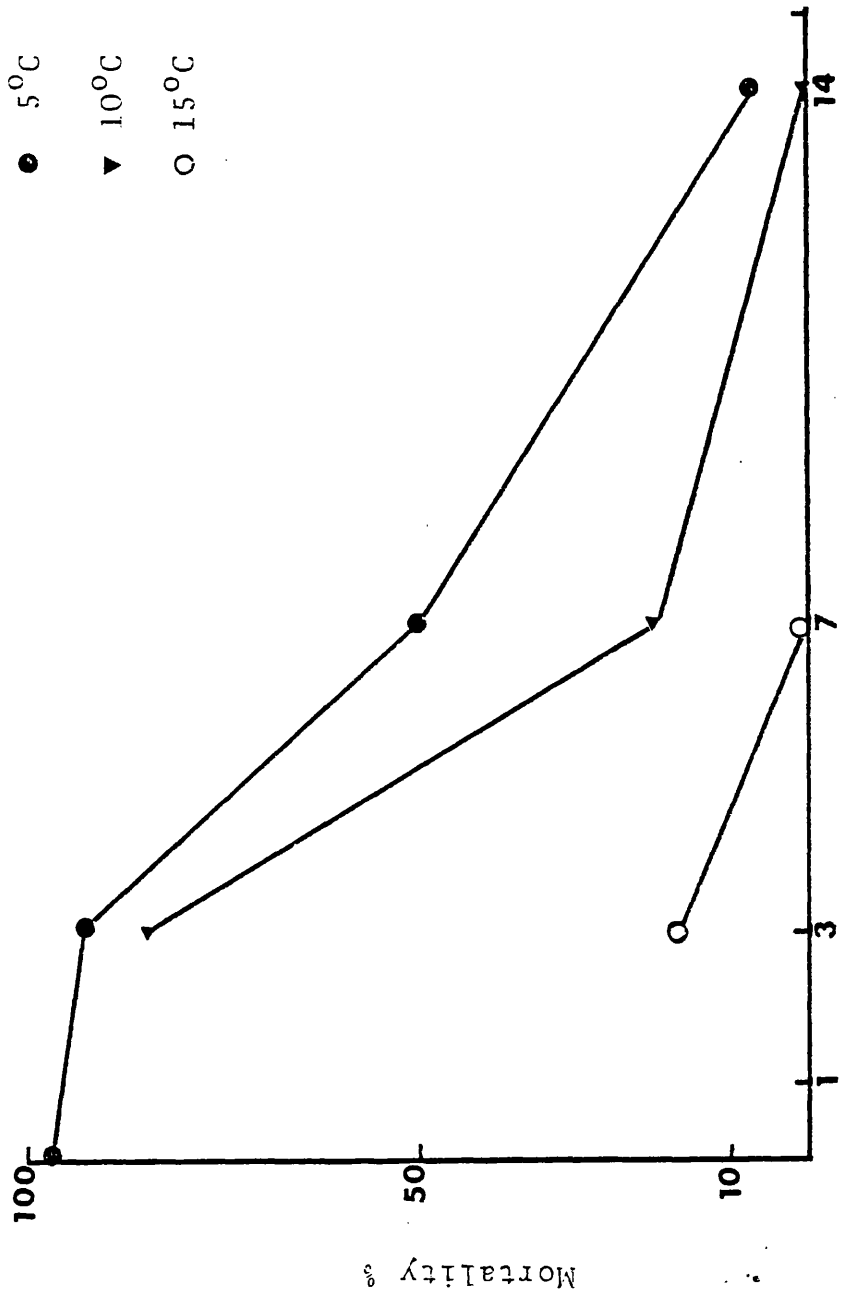

Figure 17. Effects of temperature on the persistence of fenitrothion (LD_{90})

4.14 Experiments with permethrin

The experimental procedure was similar to that as described under 4.12 except that, because of the long persistence of permethrin at low temperatures , the effect of a relatively high temperature of 30°C was also investigated. The immediate bioassay of an LD_{50} of permethrin gave an initial mortality of 82%. The possible reason for aberrant results such as this are discussed in Section 6. After a storage of 3, 10, 15 and 21 days at a temperature of 5°C, the mortalities recorded were 40%, 30% and 50% respectively. As a result of immediate feeding with a LD_{90}, the mortality recorded was 73%, storage for 3, 10, 15 and 21 days at 5°C showed mortalities of 80%, 70%, 50% and 50% respectively.

10°C storage of LD_{50} for 3, 10, 15 and 21 days showed mortalities of 60%, 58% and 70% respectively. When LD_{90} dosages were stored at 10°C for 3, 10, 15 and 21 days the mortalities recorded were 76%, 70%, 80% and 75% respectively.

Storage of LD_{50} at 15°C for 3, 7, 10, 15 and 21 days gave mortalities of 83.3%, 60%, 45%, 25% and 57.5% respectively. Whereas storage of LD_{90}, at the same temperature and for the same period of time as described above, gave mortalities of 86.6%, 75%, 50%, 37.5% and 57.5% respectively.

At 30°C, the storage of LD_{50} for 2, 10, 21 and 30 days gave mortalities of 65%, 52.5%, 62.5% and 30% respectively. The storage of LD_{90} for the same period of time and at the same temperature gave mortalities of 85%, 67.5%, 75% and 50% respectively.

Table 9. The effects of temperature on the persistence of permethrin

Time Period	Temperature 5°C		Temperature 10°C		Temperature 15°C		Temperature 30°C	
	LD_{50} 36.8 µg/g	LD_{90} 47.9 µg/g	LD_{50} 36.8 µg/g	LD_{90} 47.9 µg/g	LD_{50} 36.8 µg/g	LD_{90} 47.9 µg/g	LD_{50} 36.5 µg/g	LD_{90} 47.9 µg/g
	Mortality %		Mortality %		Mortality %		Mortality %	
Nil	82	73	82	73	82	73	82	73
2 days	-	-	-	-	-	-	65	85
3 days	40	80	60	76	83.3	86.6	-	-
7 days	-	-	-	-	-	-	60	75
10 days	30	70	58	70	45	50	52.5	67.3
15 days	50	50	70	80	25	37.5	-	-
21 days	50	50	70	75	57.5	57.5	62.5	75
30 days	-	-	-	-	-	-	30	50

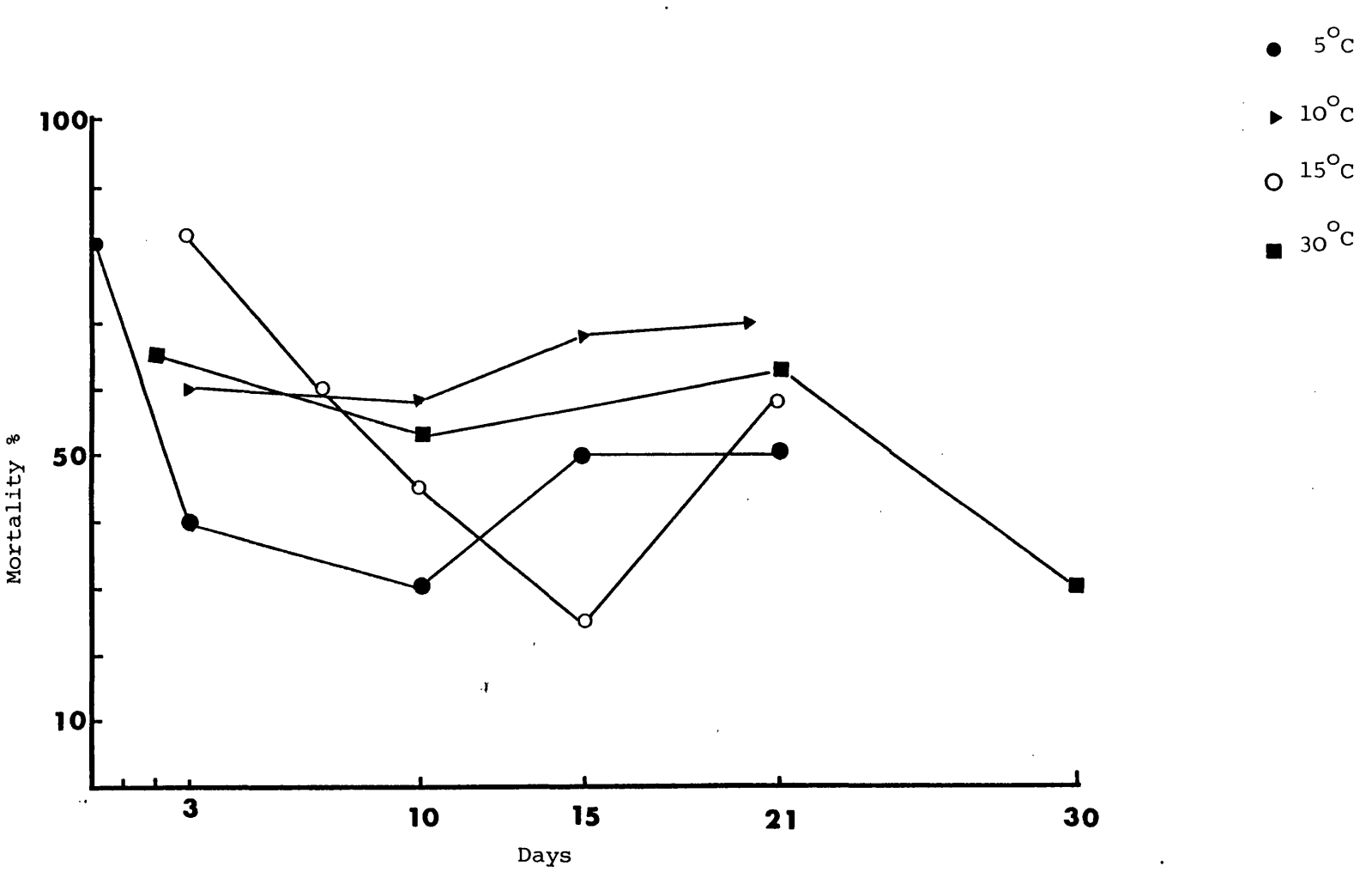

Figure 18. The effects of temperature on the persistence of permethrin (LD_{50}).

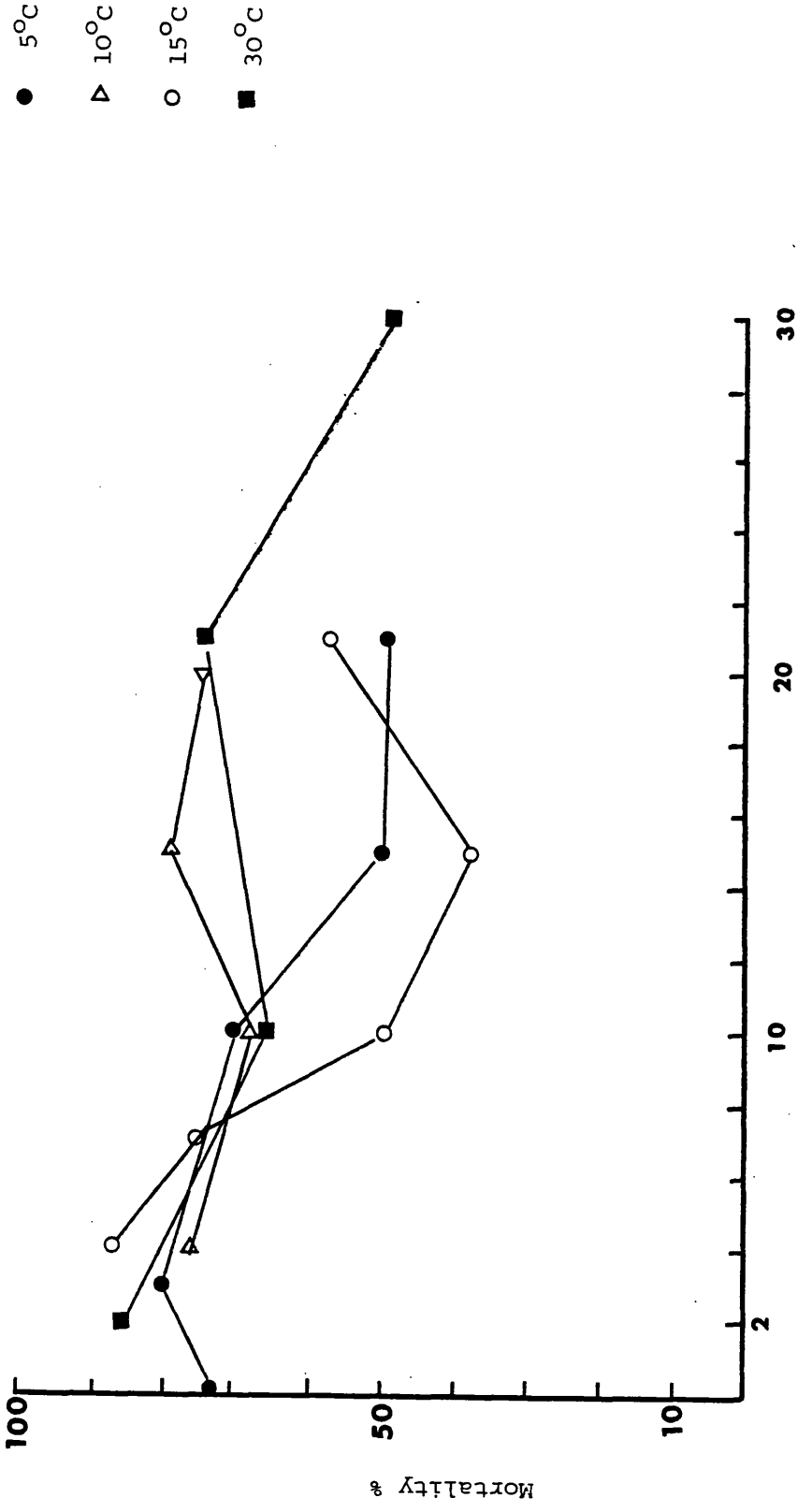

Figure 19. Effects of temperature on the persistence of permethrin (LD_{90}).

4.15 Colorimetric determination of fenitrothion residues

In an initial series of experiments, several different inert materials were tested, to determine how the material chosen for the feeding experiments (soft tissue paper) compared with other materials, including ,thin layer chromatography plates (TLC), filter paper, and "Kimwipe" tissue paper. These were also compared to a standard, in which no inert material was used, the insecticide being placed directly in the Getz tubes. Calibration curves were determined for each material after treatment with a range of fenitrothion dosages, which were measured by a pipette. The residue was then extracted with acetone, treated as described in Section 2.9 and absorbance of the final solution determined. The standard was not extracted, but used directly.

The results showed that the "Kimwipe" tissue gave the best results in that it gave the highest absorbance of material tested, although soft tissue paper gave absorbance almost as high, that is 9.5 nm when treated with 14 µg (Figure 20), so this material was used for all the subsequent experiments. In the next series of experiments, different doses of fenitrothion were placed on soft tissue paper rectangles,using the pipettes, the papers were then kept at 10°C, 15°C, 20°C, and 25°C for 72 hours, after which the residues were extracted and absorbance values determined.

The results showed that the recovery of the residues, in respect of the amount initially applied, decreased during the 72 hours period. For example, the rate of degradation at 10°C during the 3 day period was 2.3% at all dosage rates. At the higher temperature of 25°C the

loss was 70% during the same period. This indicates that at increased temperatures the rate of loss appears to be considerably less than that found by bioassay techniques.

Table 10 shows the results obtained by using different inert materials for the determination of fenitrothion residues. It can be seen that when fenitrothion was used directly for the optical measurement, the parameter of coefficient "r^2" was 0.98, the parameter of intercept "a" was 0.31, and the parameter of slope obtained was "b" = 0.19. By using TLC plates the parameters were 0.92, 1.27, and 0.05 respectively. By using "kimwipe" tissue paper the values for the parameters were 0.95, 2.16, and 0.74 respectively. By using "soft tissue paper" the values for the parameters "r^2", "a", and "b" obtained were 0.98, 3.14 and 0.47 respectively. Calibrated graphs for all the materials used for the study are presented in Figure 20.

Table 11 shows the summarised results of the residues obtained through the effect of temperature on the persistence of fenitrothion. It can be observed from this table that the insecticide treated soft tissue paper,when stored for three days at $10^{\circ}C$, gave parameters for "r^2", "a", and "b" of 0.96, 1.77, and 0.46 respectively. Three days storage of insecticide treated soft tissue paper at $15^{\circ}C$ resulted in 0.99, 1.84, and 0.37 as the values for the parameters of "r^2", "a" and "b" respectively. Storage of the insecticide treated soft tissue paper at $20^{\circ}C$ for three days gave values of 0.99, 1.42, 0.29 as the parameters of "r^2", "a" and "b" respectively. When stored at a temperature of $25^{\circ}C$ for the same period, the values obtained for the parameters were 0.99, 0.87 and 0.14 respectively. Figure 21 shows the results obtained.

Table 10. Results obtained by colorimetric determination of fenitrothion by using different inert materials.

Material	Concentration (µg)	Absorbance	r^2	a	b
Standard	2	0.7	0.98	0.31	0.19
	5	1.4			
	10	1.9			
	15	3.3			
	20	4.1			
T.L.C. plates	2	1.2	0.92	1.27	0.05
	5	1.6			
	10	1.8			
	15	1.9			
	20	2.2			
Kimwipe tissue paper	2	4.1	0.95	2.16	0.74
	5	5.1			
	10	9.8			
Soft tissue paper	2	4.1	0.98	3.14	0.47
	6	5.7			
	10	8.3			
	14	9.5			

Table 11. Evaluation of the effects of temperature on the persistence of fenitrothion (72 hours)

Concentration (µg)	Temp.	Absorbance	r^2	a	b
2	10°C	2.6	0.96	1.77	0.46
6		5.0			
10		5.7			
14		8.5			
2	15°C	2.7	0.99	1.80	0.37
6		3.8			
10		5.8			
14		6.9			
2	20°C	1.9	0.99	1.42	0.29
6		3.3			
10		4.2			
14		5.4			
2	25°C	1.1	0.99	0.87	0.14
6		1.7			
10		2.3			
14		2.7			

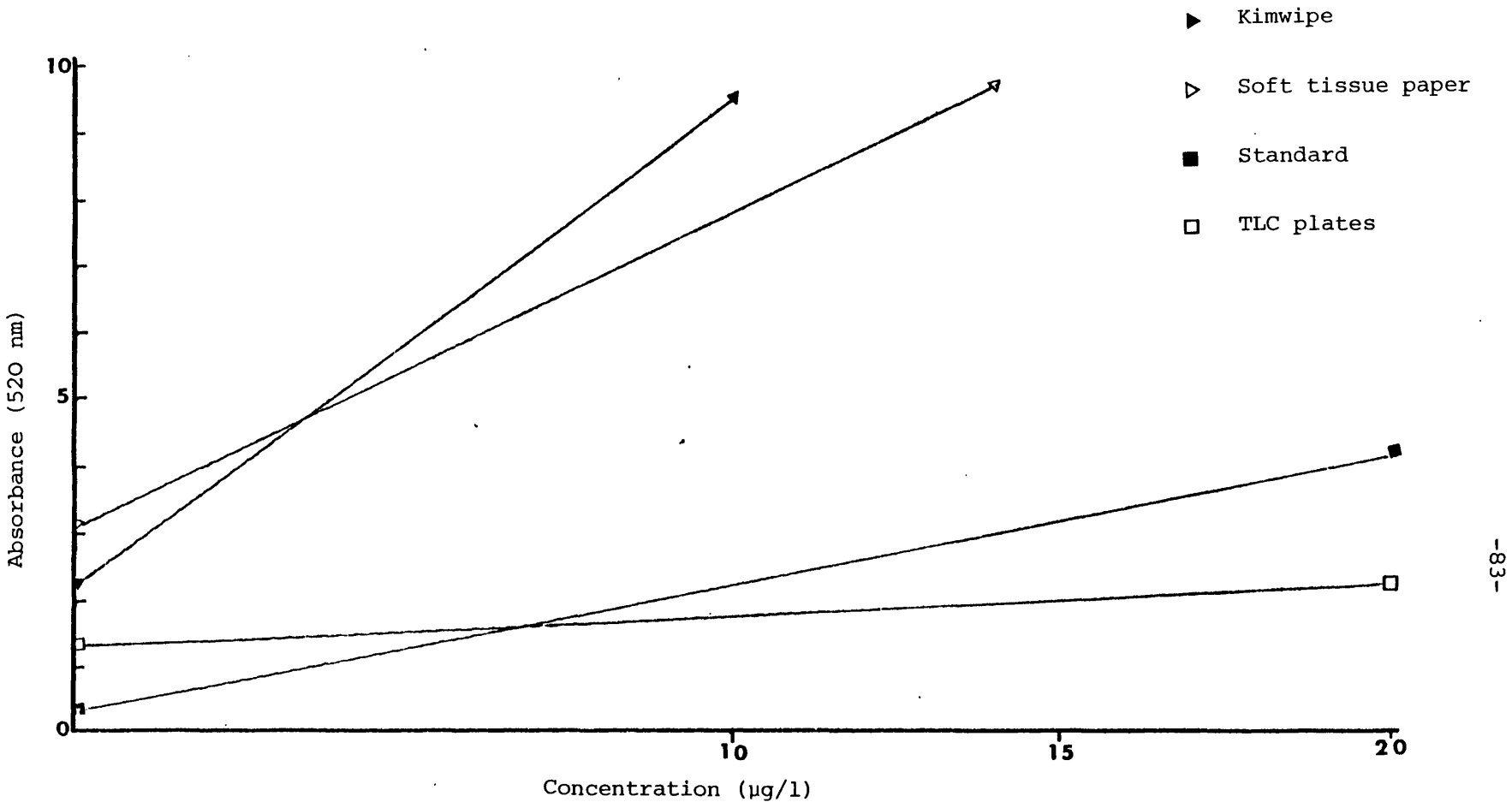

Figure 20. Determination of fenitrothion residues.

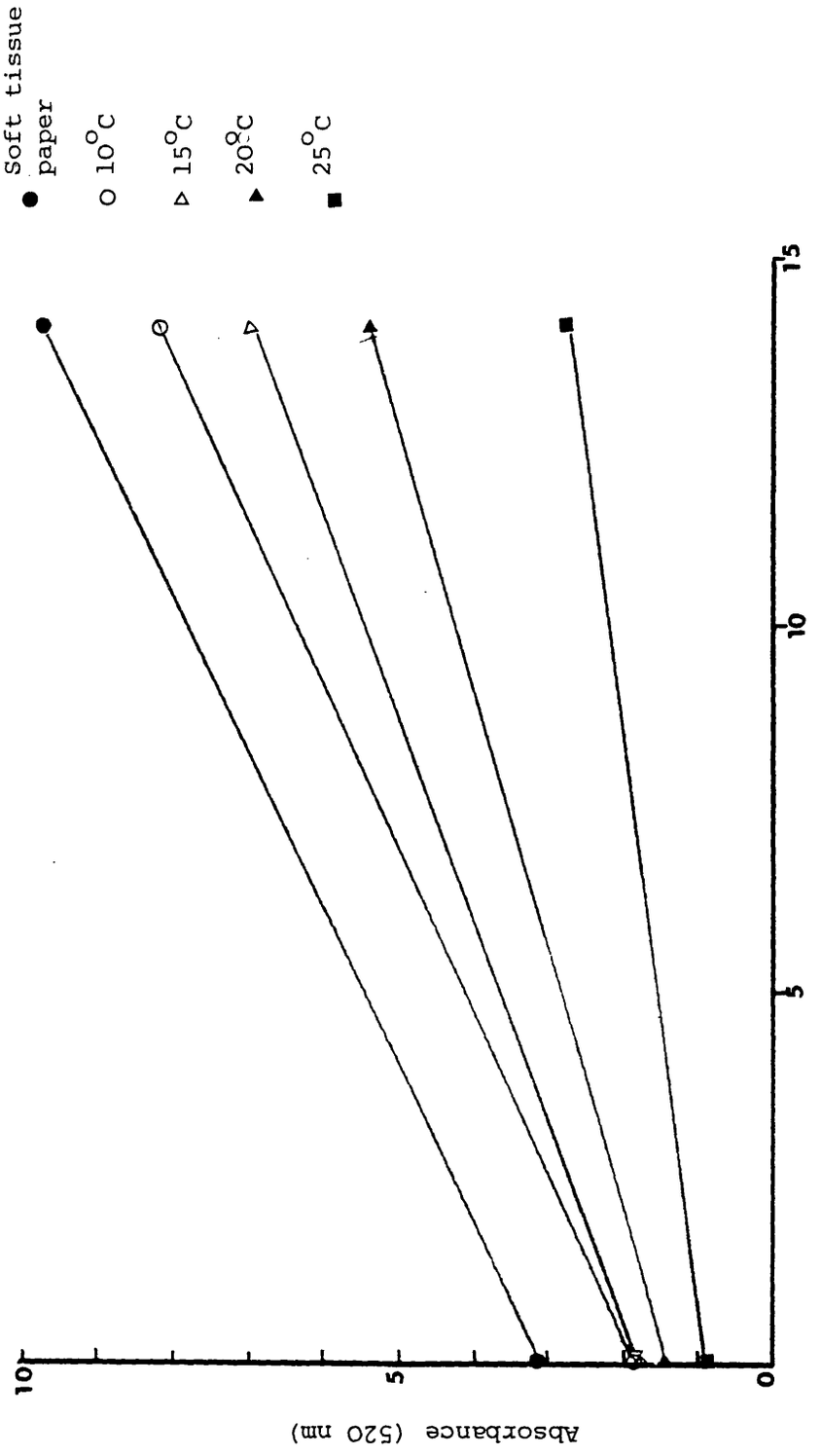

Figure 21. Determination of fenitrothion residues.

4.16 Summary and Discussion

The results show that permethrin residues retained their toxicity to 4th. instar locusts at all temperatures tested for at least 21 days. It is unfortunate that the experiments had to be terminated at this point as all the treated tissue paper had been utilized. These results agree with those of Hadaway *et al.* (1977), who on a bioassay with Tsetse Flies showed that, when permethrin sprayed ivy leaves were kept at 25°C and 50 - 55% RH, there was no loss of residual toxicity of the insecticide during a period of 29 days.

Fenitrothion, on the other hand, was less persistent over the range of temperatures from 5°C - 15°C, for most of the activity had been lost after seven days. The results corroborate those of Lemon (1967) who, on a comparative study with bromophos, malathion and fenitrothion against ten species of stored products insects, found that fenitrothion was moderately persistent (1 - 2 weeks).

With lindane, however, no insecticidal activity was detectable at either temperature with LD_{50} or LD_{90} after seven days; after three days the activity was very greatly reduced, although this insecticide is normally regarded as a moderate "persistent hydrocarbon insecticide". The possible reason for lindane's short persistence could be because of the high volatilic nature of the insecticide. Vaporization rates of insecticides such as lindane, probably account for the very rapid disappearance of deposits (Ebeling, 1963; Gunther, 1969; Quraishi, 1977).

The results on the determination of fenitrothion residues indicated that there is a difference in the rate of loss of fenitrothion residues by the two methods, namely bioassay and chemical determination. It seems possible that it is a difference more apparent than real. The bioassay measures the insecticidal activity of the residue, but if fenitrothion breaks down to non-toxic residues or is present at sub-lethal levels, then no activity will be shown. On the other hand, such residues would be detectable by the chemical technique.

4.2 Effects of light on persistence

4.21 Introduction

Sunlight (natural day light) as received on the surface of the earth is made up of mostly infra red and the visible region of the spectrum (Henderson and Marsden, 1972; Williams, 1962; Calverts and Pitts, 1966). To simulate this under laboratory conditions many different kinds of electric lamps have been used, such as Mercury arcs, Carbon arcs, Xeron Resonance Lamps, and Fluorescent lamps. Probably the closest to natural light is the North Light/Colour Matching fluorescent tube, which was used in the present work. The colour characteristic, or spectrum of this fluorescent tube is shown in Figure 1 and is compared with natural day light (Cox, 1976). For this work the tissue paper method of feeding was used (see Sections 2.4 and 4.1).

Again two rates of dosage for each unit of tissue paper were used, i.e. the LD_{50} and LD_{90}, applied by microcapillary tube (Section 2.3). After dosing, a check was made using 4th. instar locusts that the doses approximated to the LD_{50} and LD_{90} and the rest of the treated papers

were then placed in an incubator maintained at 5°C and illuminated by six North Light tubes as described in Section 2.6. After the tissue paper rectangles had been in the incubator for the required length of time, they were removed and immediately moistened with two drops of 0.125 molar solution of sucrose and fed to the pre starved test insects in the standard manner.

4.22 Experiments with lindane

In an earlier section it was shown that lindane did not persist for more than three days at a temperature of 5°C in the absence of light (Section 4.12). So it was considered unlikely that, at the same temperature and in the presence of light, any persistence would be detectable at all. A small experiment was therefore conducted to confirm this. Tissue papers were dosed with the LD_{50} and the LD_{90} levels, then fed to fourth instar hoppers immediately after the treatment; the mortalities obtained were 60% and 90% respectively. Papers kept at 5°C in the incubator and exposed to fluorescent lights for three days gave mortalities of 2.5%, and 5.0% for the LD_{50} and LD_{90} respectively. As expected lindane did not persist for as long as three days on tissue paper at 5°C, in the presence of light.

4.23 Experiments with fenitrothion

Tissue papers were dosed with the LD_{50} and LD_{90} levels according to the standard procedure and some immediately fed to 4th. instar locusts. The mortalities after 24 hours were 60% and 95% respectively. After storage, the mortality among the group fed the LD_{90} level fell gradually,

until after twenty days there was little or no detectable mortality (see Table 12 and Figure 22). For the LD_{50} tissue papers, however, the mortality increased slightly after one day to 77.5%, but then fell gradually until no mortality occurred after ten days (Table 12 and Figure 22).

4.24 Experiments with Permethrin

Similar experiments were carried out with permethrin to the ones described for fenitrothion. The results showed that the application of LD_{50} and LD_{90} as a check gave initial mortalities of 70% and 75% respectively. At both dosage levels, mortality at first fell after three days, but at subsequent determinations, the pattern was irregular as shown clearly by reference to figures 23. After 56 days the LD_{90} dose still gave a mortality of 65% and the LD_{50} one of 50%. (Table 13 and Figure 23). These experiments were carried out for a much longer period than the temperature ones because of the prolonged persistence of permethrin shown by those experiments.

4.25 Summary and Discussion

These results, therefore, confirm the short persistence of lindane, the intermediate persistence of fenitrothion and the prolonged persistence of permethrin. When the effects on persistence of temperature are compared and contrasted with the effects of light, it can be seen that comparing only those experiments done at $5^{o}C$, there was very little difference. Although the added effects of light might have been expected to be cumulative it appears to make little difference. So, the 'half-life' of fenitrothion with temperature alone was 4 - 6 days,

with temperature plus light it was 4 - 5 days. With permethrin, temperature alone 12 - 15 days and temperature plus light 14 - 15 days.

In this experiment, however, the persistence of permethrin was continued for a longer period than in the experiments involving temperature only. The results showed that persistence of permethrin was not only detectable after 56 days, but even by this time was only reduced by a small percentage. However, loss of persistence, as seen from the graph on days 7 - 20 is anomolous, and is to some extent also shown by the LD_{50} graph at $5^{o}C$ (Figure 18). It is difficult to explain the nature of the irregular pattern of mortalities except by assuming it is due to variability in susceptibility of the test insects to an insecticide that persists with little, if any, loss of toxicity, for at least 56 days.

Table 12. Effect of light on the persistence of fenitrothion.

Time period	Temperature	Light Intensity	Photoperiod	Mortality % LD_{50}	Mortality % LD_{90}
Nil	5 ± 1°C	2415.6 lux	16hrs D/8hrsN	60.0	90.0
1 day	"	"	"	77.5	90.0
4 days	"	"	"	47.5	87.5
6 days	"	"	"	33.3	75.0
10 days	"	"	"	0	45.0
13 days	"	"	"		52.5
20 days	"	"	"		5.0

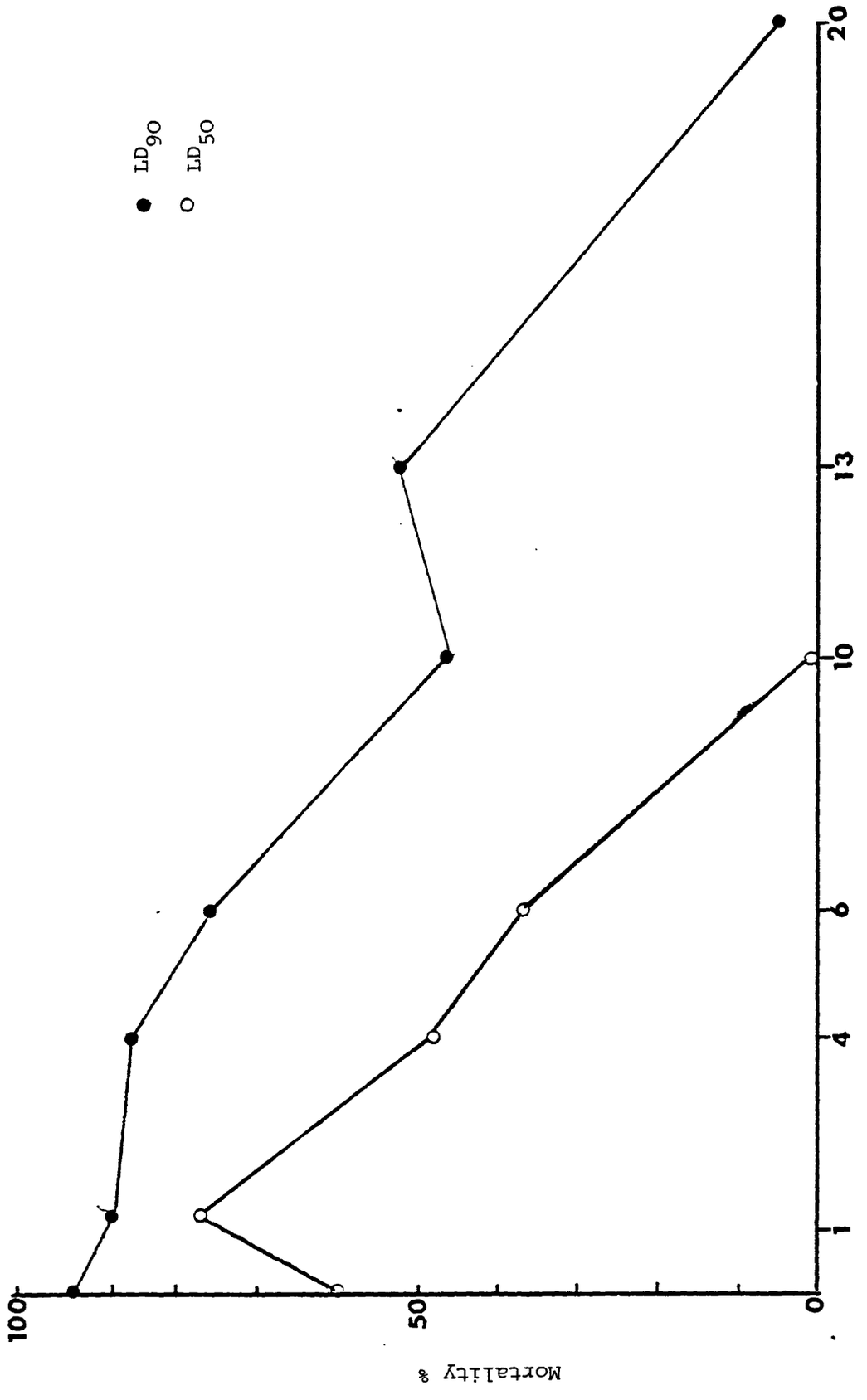

Figure 22. Effects of light on fenitrothion's persistence

Table 13. Effects of light on the persistence of permethrin.

Time period	Temperature	Light Intensity	Photoperiod	Mortality % LD_{50} 36.8 µg/g	Mortality % LD_{90} 49.9 µg/g
Nil	5 ± 1°C	2415.6 lux	16hrs D/8hrs N	75.0	75.0
3 days	"	"	"	70.0	86.6
7 days	"	"	"	45.0	45.0
10 days	"	"	"	60.0	55.0
15 days	"	"	"	30.0	50.0
21 days	"	"	"	70.0	82.5
56 days	"	"	"	50.0	65.0

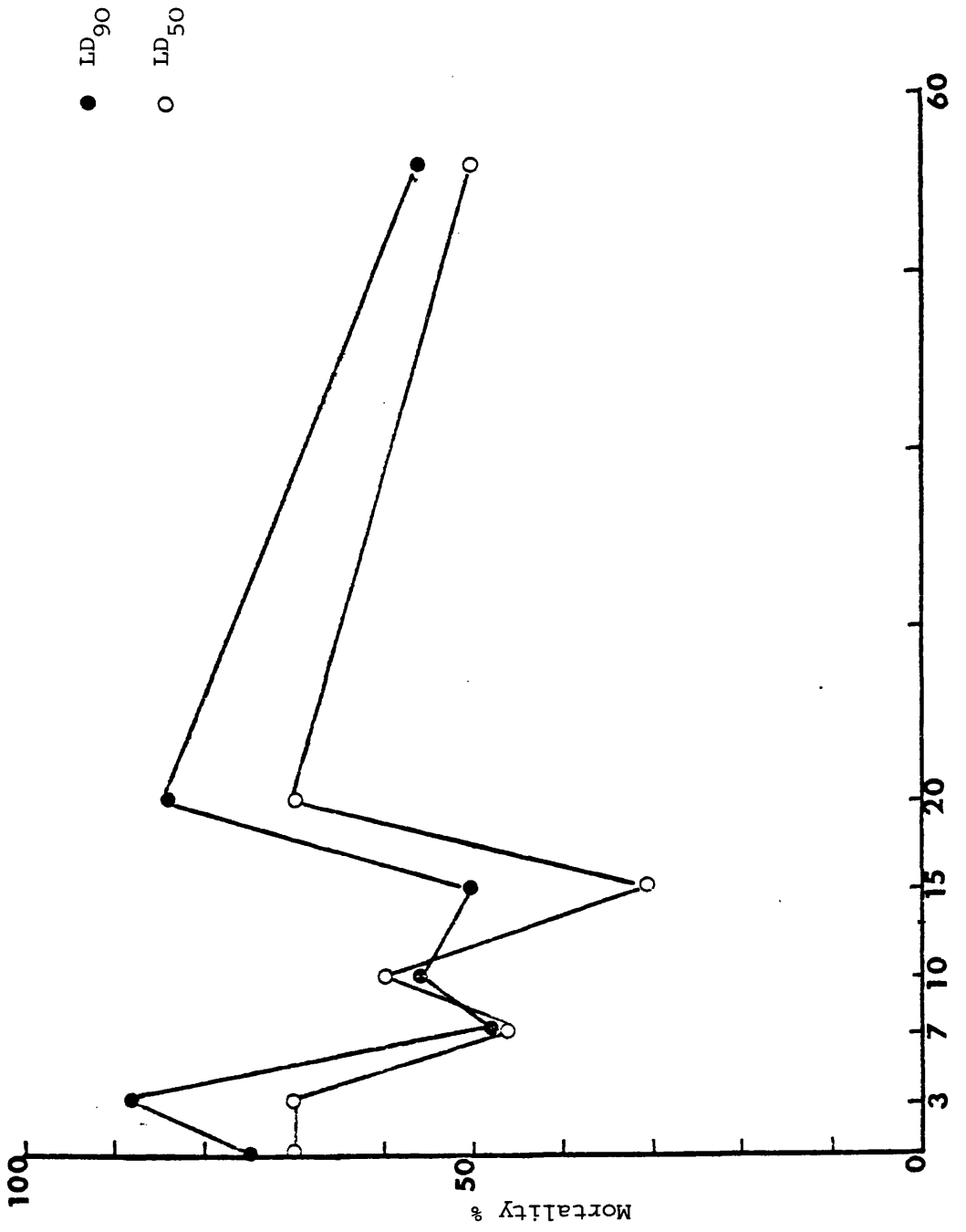

Figure 23. Effects of light on permethrin's persistence

5.0 Persistence of insecticide on growing plants

In earlier experiments, tissue paper was used as the substrate as it was an inert material with no interaction or breakdown with the chemical insecticides. It was therefore a convenient substrate for evaluating the different effects of the various physical factors. But tissue paper is an artificial medium and has little relevance to field conditions. In this section, therefore, growing plant material was used as described in Introduction (Section 1). Choice of plant material is important in this experimental work because of the differences of leaf structure (i.e. presence of hairs, or wax layers, fleshy leaves etc.) that may affect insecticidal persistence. The plants chosen were:-

1) Privet *(Ligustrum ovalifolium)*
2) Wheat *(Triticum* sp.)
3) Brussels sprouts *(Brassica olearacea gemmifera)*

Privet is a woody plant with a pronounced wax layer on the leaves. Wheat, the only Monocotyledonous plant used, has a thin bladed, narrow, hairy leaf with very little wax; it is of economic importance in locust areas. Brussels sprouts have a wide leaf, with an intermediate wax coating and no hairs. Details of growth procedures were outlined in Section 2.7. The experimental procedure was comparable to that for tissue paper, but only two insecticides, namely, fenitrothion and permethrin were used. Lindane was not used because its very low persistence made it unlikely to produce interesting results, also time and availability of locusts were both severely limited. Furthermore,

in view of the limited time available, only the LD_{90} was used for fenitrothion, and the LD_{50} for permethrin. The individual doses of insecticides were measured with the graduated microcapillary and applied directly to the leaf surfaces. It was noticeable that on wheat the applied dose spread longitudinally for a small extent, but on privet it remained as a discrete spot and on Brussels sprout is appeared to be absorbed into the leaf with limited spread. The actual site of application was marked by waterproof ink to allow for subsequent detection. After dosing, the treated plants were kept in the incubator for the required period of time at $5^{o}C$ and 2415.6 lux of light intensity.

5.1 Effects of light and temperature

These two factors, namely light and temperature, were combined in this experiment because:-

1) It has been shown (Section 4.2) that light has very little effect on persistence;
2) The plants needed some light for satisfactory photosynthesis and growth.

So, the temperature used was $5^{o}C$ and the light was derived from the same arrangement of North Light lamps as in section 2.6, 4.2).

5.12 Experiments with fenitrothion

The LD_{90} was applied on wheat leaves and when fed immediately, the mortality recorded was 100%. The remainder of the treated plants were kept

in an incubator and fed to the fourth instar locusts after 4, 10, 15 and 21 days. The recorded mortalities were 75%, 50%, 20% and 0% respectively (Table 14 and Figure 24).

When LD_{90} dose was applied on Brussels sprout leaves and fed immediately, the mortality observed was 100%. After the incubation period of 4, 10, 15 and 21 days, the mortalities recorded were 82.5%, 52.5%, 5% and 0% respectively (Table 14 and Figure 24).

Mortality recorded on privet leaves as a result of immediate feeding was 90%, the rest of the treated plants were kept in the incubator for 4, 10, 15 and 20 days. When fed to 4th. instar locusts, mortalities obtained were 80%, 42.5%, 15% and 0% respectively (Table 14 and Figure 24).

5.13 Experiments with permethrin

In this work the doses used were LD_{50}. When this dose was applied to wheat leaves and fed immediately to 4th. instar locusts, the mortality recorded was 55%, and the rest of the treated plants were kept in the incubator for 10, 20, 30 and 40 days, and the mortalities recorded were 45%, 72.2%, 80% and 85% respectively.

When the LD_{50} dose was applied to Brussels sprout leaves and fed immediately to the locusts, the mortality recorded was 70%. The rest of the treated plants were stored for 10, 20, 30 and 40 days. After the expiry of the required time period, the treated leaves were fed to 4th. instar locusts and the mortalities observed were 60%, 40%, 67.5% and 35% respectively.

Privet plants treated with LD_{50} were kept in the incubator for 10, 20, 30, and 40 days. Prior to the transfer of the treated plants, some of the leaves dosed with the insecticide were fed to the locusts immediately, giving a mortality of 80%. After the required period the treated plants were taken out of the incubator and fed to the 4th. instar locusts. The mortalities recorded were 82.5%, 75%, 85% and 57.5% respectively, (Table 15 and Figure 25).

5.14 Summary and Discussion

1. Fenitrothion

   There were no obvious differences in the role of loss of the insecticide on any of the plants used. In fact the loss is similar to that recorded in the previous experiments using tissue paper under the same light and temperature regimes.

2. Permethrin

   Although persistence was evaluated for a longer period (40 days) and the results were variable, there are indications that levels of activity are slightly higher on wheat, followed by privet and less on Brussels Sprouts at the end of the test period.

Table 14. Persistence of fenitrothion on growing plants under the light intensity of 2415.6 lux, temperature $5^{o} \pm 1^{o}C$, and photoperiod of 16 hours Day / 8 hours night.

Time Period	PLANT USED		
	Wheat	Brussels sprout	Privet
	LD_{90} 13.0 µg/g	LD_{90} 13.0 µg/g	LD_{90} 13.0 µg/g
	Mortality %	Mortality %	Mortality %
Nil	100	100	90
4 days	75	82.5	80
10 days	50	52.5	42.5
15 days	20	5.0	15.0
20 days	0	0	0

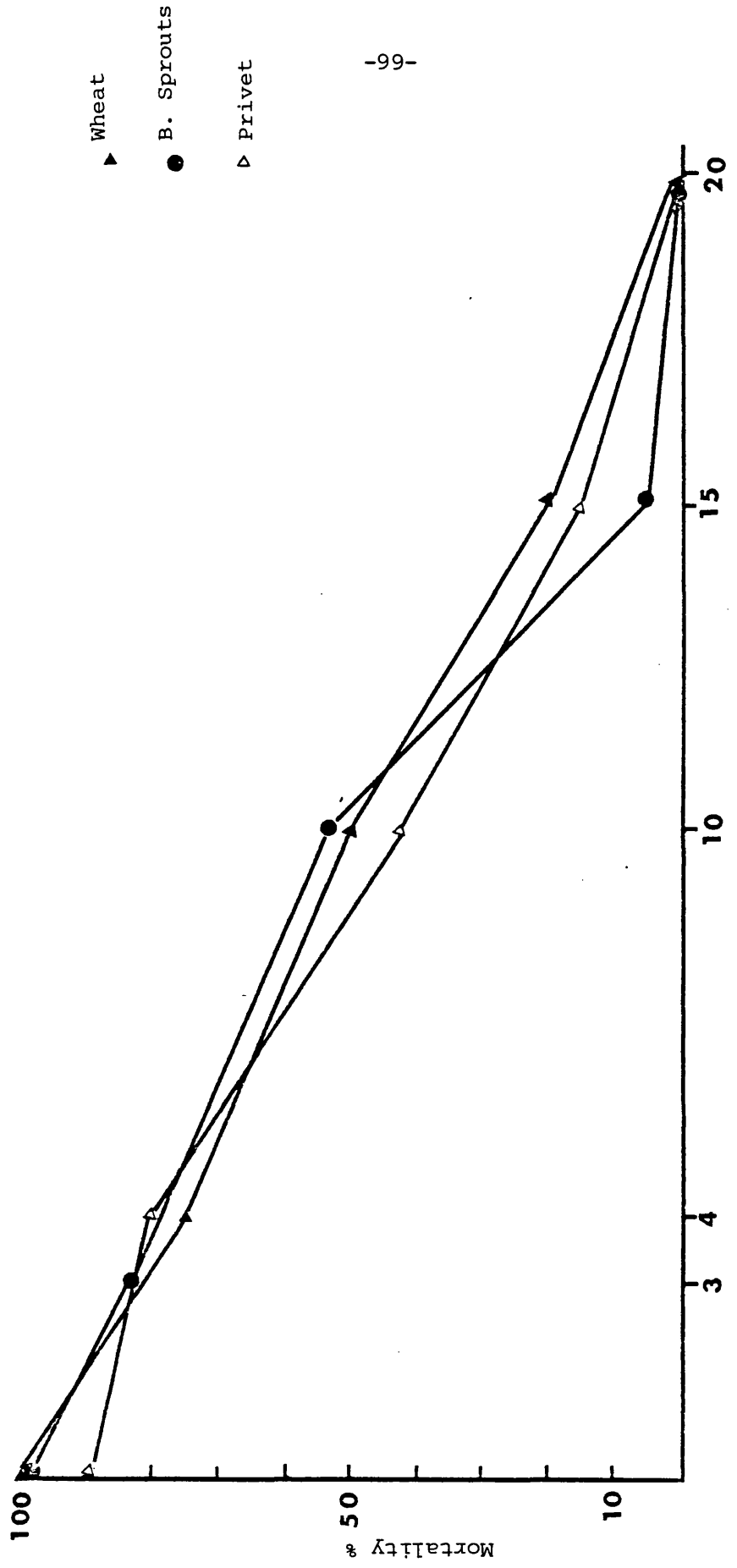

Figure 24. Persistence of fenitrothion on growing plants,

Table 15. Persistence of permethrin on different growing plants under the light intensity of 2415.6 lux, temperature 5 ± 1°C, and photoperiod of 16 hours Day / 8 hours Night

Time period	PLANTS USED		
	Wheat	Brussels sprouts	Privet
	LD_{50} 36.8 µg/g	LD_{50} 36.8 µg/g	LD_{50} 36.8 µg/g
	Mortality %	Mortality %	Mortality %
Nil	55	70	80
10 days	45	60	82.5
20 days	72	40	75.0
30 days	80	67.5	85.0
40 days	85	35	57.5

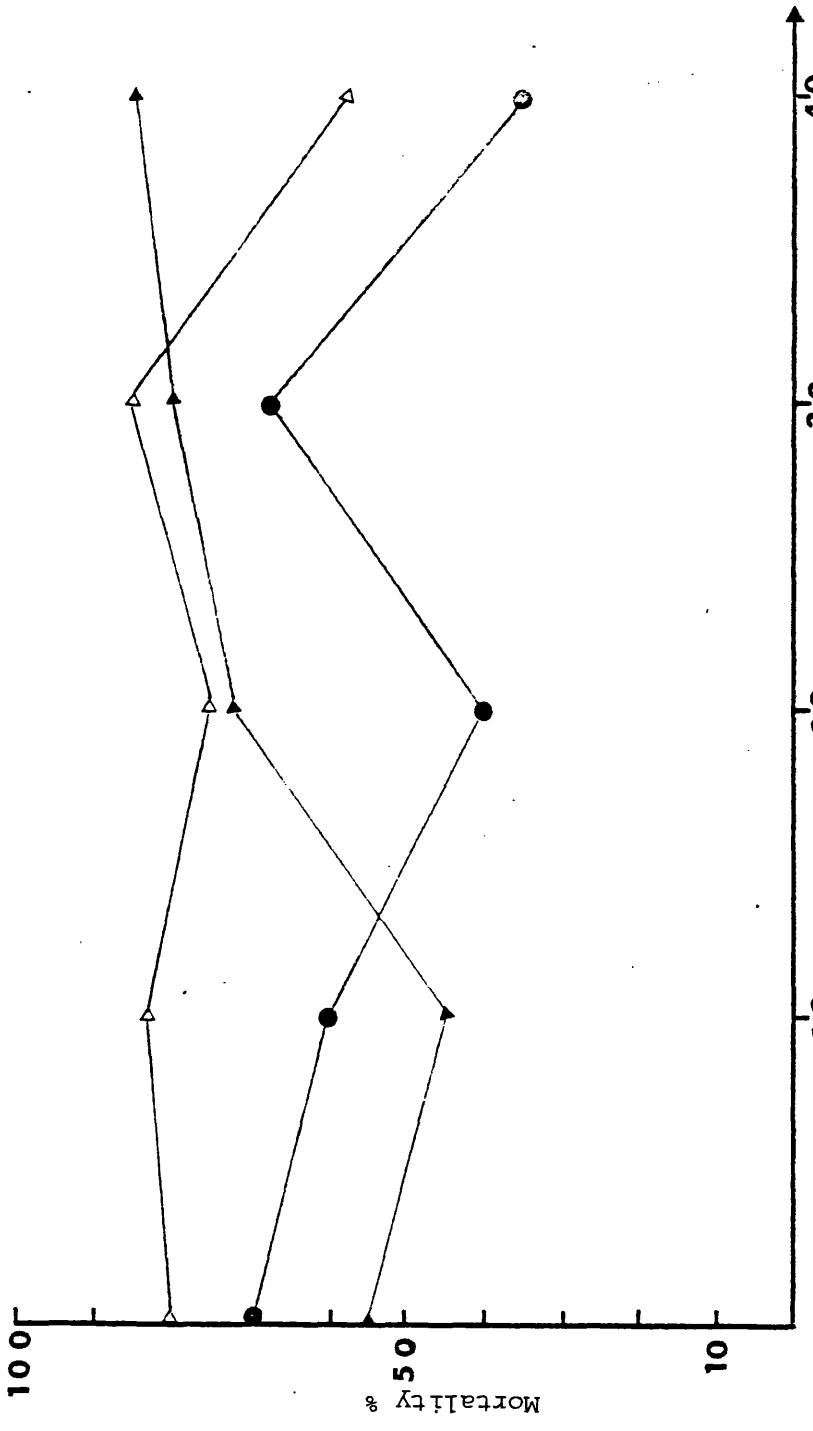

Figure 25. Persistence of permethrin on growing plants.

because experiments had not gone on long enough. However, the graphs all seem very similar.

5.2 Effects of rainfall

The effects of temperature and light have already been evaluated (Section 4.14, 4.2 and 5.13). These results showed that these two factors have little or no effect on the persistence of permethrin. So the effects of rainfall on the persistence of this insecticide on growing plants was evaluated. In this work only the LD_{50} of permethrin was used because of limitation of time.

To simulate rainfall, an overhead sprinkler in the form of a 'rose' of a watering can was used. Tbe rain water was collected in a reservoir outside the greenhouses and pumped to the sprinkler. The water droplet size was measured using the technique described by Matthews(1979) and the results are shown in Figure 26 and Table 16, which shows that the vmd was of 6.2 mm (6,200micron) and nmd of 3.2 mm (3,200 micron).

The plants used were the same as those described inSection 2.7 and 2.8, and the methods of growth and treatment were similar.

5.21 Experiments with permethrin

When LD_{50} was applied to wheat leaves and fed immediately to the test insects, the mortality recorded was 70%. After storage under a standard temperature of 5°C and light (2415.6 lux) conditions for 3, 10, 15 and 21 days and using simulated rain for a three hour period, the mortalities recorded were 87.5%, 70.0%, 65.0% and 77.5% respectively,

whereas using the simulated rain for six hours the mortalities recorded were 82.5%, 60.0%, 65.0% and 82.5% respectively.

When privet leaves, treated with LD_{50} of permethrin, were fed immediately to the test insects the mortality observed was 67.5%. After storage of 3, 10, 15 and 21 days, under the standard conditions of temperature and light and using the rain factor for three hours, the mortalities recorded were 70%, 52.5%, 42.5% and 50% respectively. Whereas when using the rain period for six hours the mortalities recorded were 37.5%, 47.5%, 22.5% and 40% respectively.

When Brussels sprouts leaves were treated with LD_{50} and fed immediately to 4th. instar prestarved hoppers, the mortality recorded was 55%. When stored at the standardised temperature and light conditions for 3, 10, 15 and 21 days and kept under the overhead sprinkler for three hours, the mortalities recorded were 20%, 65%, 17.5% and 10% respectively, whereas a six hours rain period gave mortalities of 55%, 15%, 45% and 20% respectively.

All these results are presented in Table 17 and Fwgures 27 and 28.

5.22 Summary and Discussion

Despite the variability of the mortality levels in the results, there is an indication that the effects of rain on the persistence of permethrin, vary according to the substrate. On wheat there is little, if any, loss of permethrin throughout the 21 day period. The loss is greater on privet specially with six hour rain regime, but most loss

occurred on Brussels sprouts where mcrtality dropped to 10% and 20% after 21 days.

These differences between plants could be attributed to the nature of their leaf surfaces. Both privet and Brussels sprouts leaves have a relatively thick layer of epicullticular wax compared with the leaves of wheat. The permethrin deposits is likely to be more subjected to being "washed off" on leaves with a thick wax layer.

Table 16. Calculations for the water droplets determination of nmd and vmd.

True Size "d"	Number "N"	% N	Σ% N	d^3	Nd^3	% N d^3	Σ% Nd^3
1.0	18	15.9	15.9	1	18.0	0.17	0.17
1.5	7	6.1	22.0	3.3	23.6	0.22	0.40
2.0	11	9.7	31.8	8.0	88.0	0.82	1.20
2.5	5	4.4	36.1	15.6	78.0	0.73	1.90
3.0	18	15.9	52.0	27.0	486.0	4.53	6.50
3.5	4	3.5	55.5	42.8	171.5	0.16	6.60
4.0	8	7.0	62.5	64.0	512.0	4.80	11.43
4.5	1	0.8	63.3	91.1	91.1	0.85	12.28
5.0	16	14.1	77.1	125.0	2000.0	18.65	30.93
5.5	2	1.7	79.1	166.4	332.0	3.09	34.02
6.0	6	5.3	84.4	216.0	1296.0	12.0	46.02
6.5	3	2.6	87.0	274.6	823.9	7.7	53.72
7.0	14	12.3	99.3	343.0	4802.0	44.8	98.52
Total	113				10722.2		

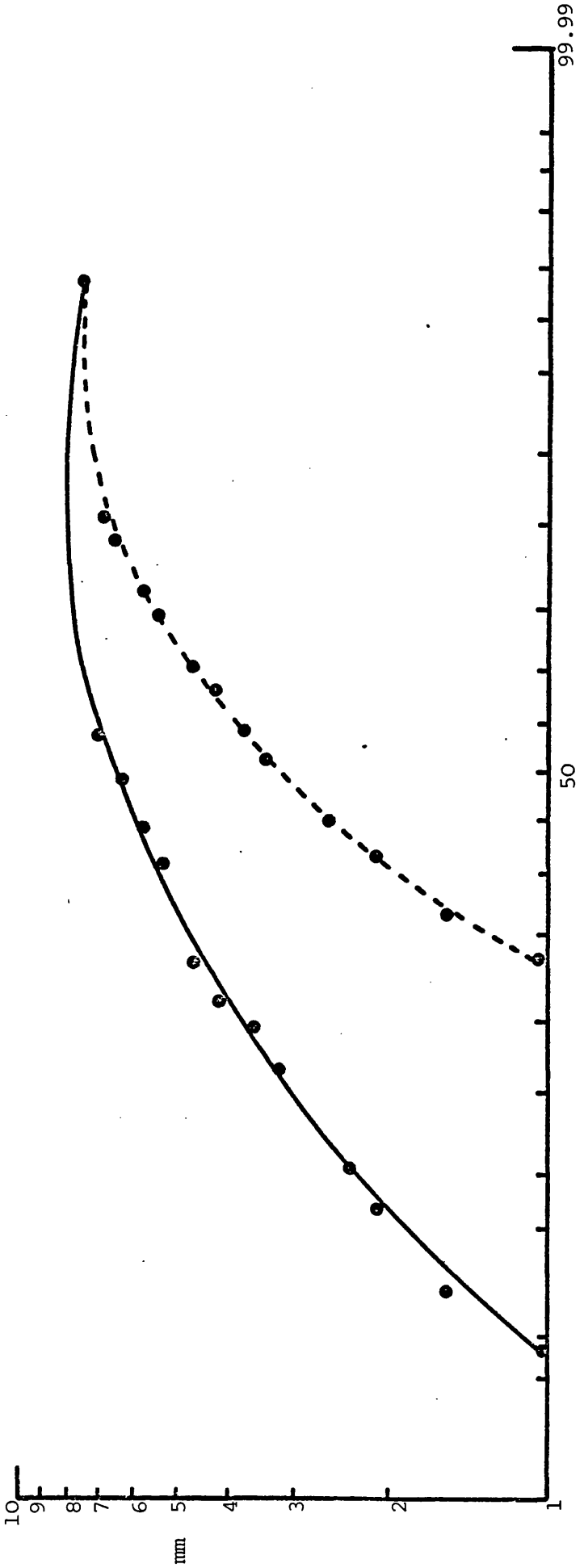

Figure 26. Graph of cumulative percentage against mean droplet size to calculate the volume (solid curve) and nmd (broken curve).

Table 17. The effects of rainfall on the persistence of permethrin on wheat, privet and Brussels sprouts

Time period	Wheat		Privet		Brussels sprouts	
	LD_{50} 36.8 µg/g		LD_{50} 36.8 µg/g		LD_{50} 36.8 µg/g	
	Rain period		Rain period		Rain period	
	3 hrs	6 hrs	3 hrs	6 hrs	3 hrs	6 hrs
	Mortality %		Mortality %		Mortality %	
0 days	70.0	70	67.5	67.5	55.0	55.0
3 days	87.5	82.5	70.0	37.5	20.0	15.0
10 days	70.0	60.0	52.5	47.5	65.0	45.0
15 days	65.0	65.0	42.5	22.5	17.5	25.0
21 days	77.5	82.5	50.0	40.0	10.0	20.0

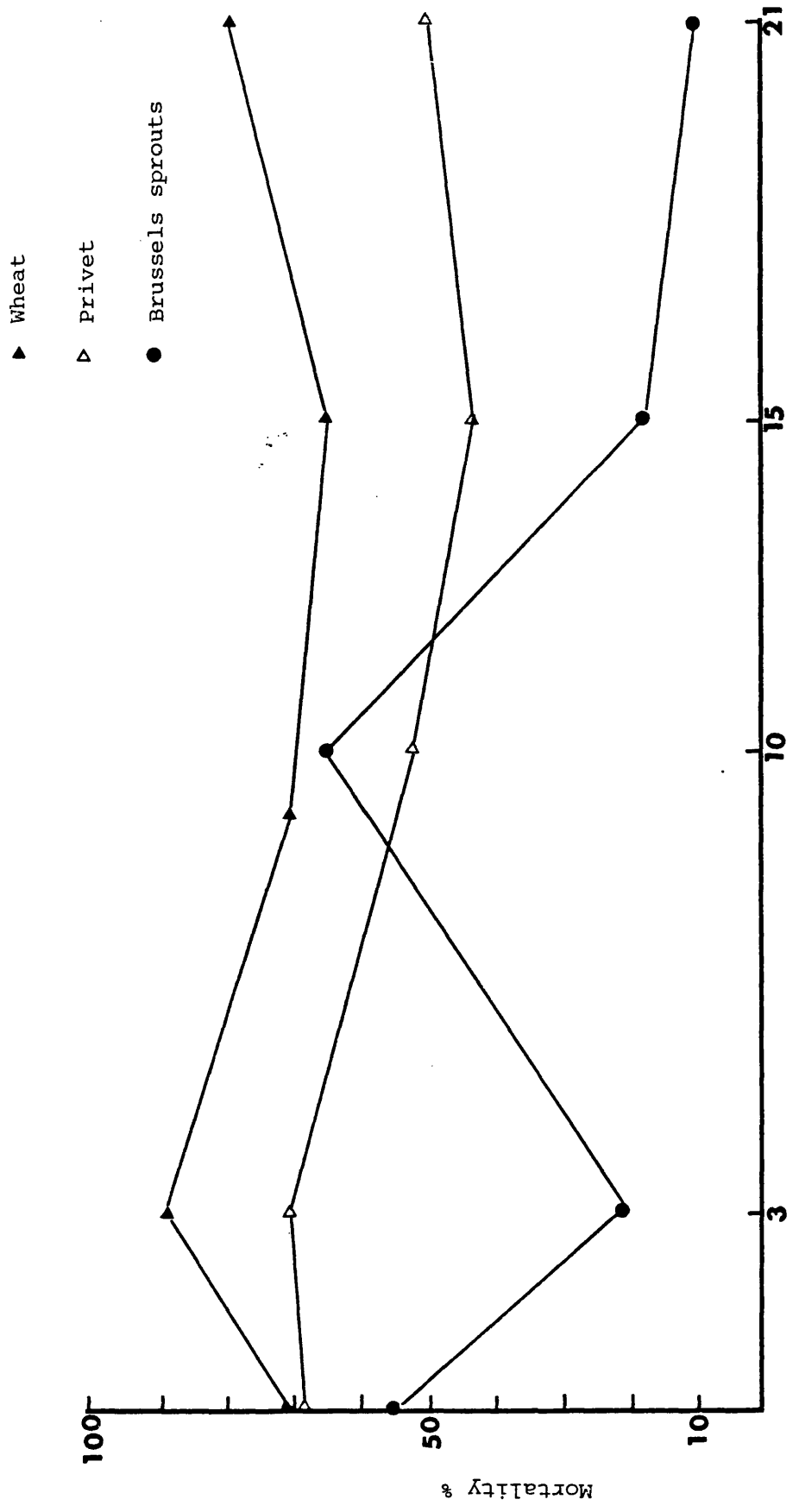

Figure 27. Effects of rainfall (3 hrs) on the persistence of permethrin on growing plants.

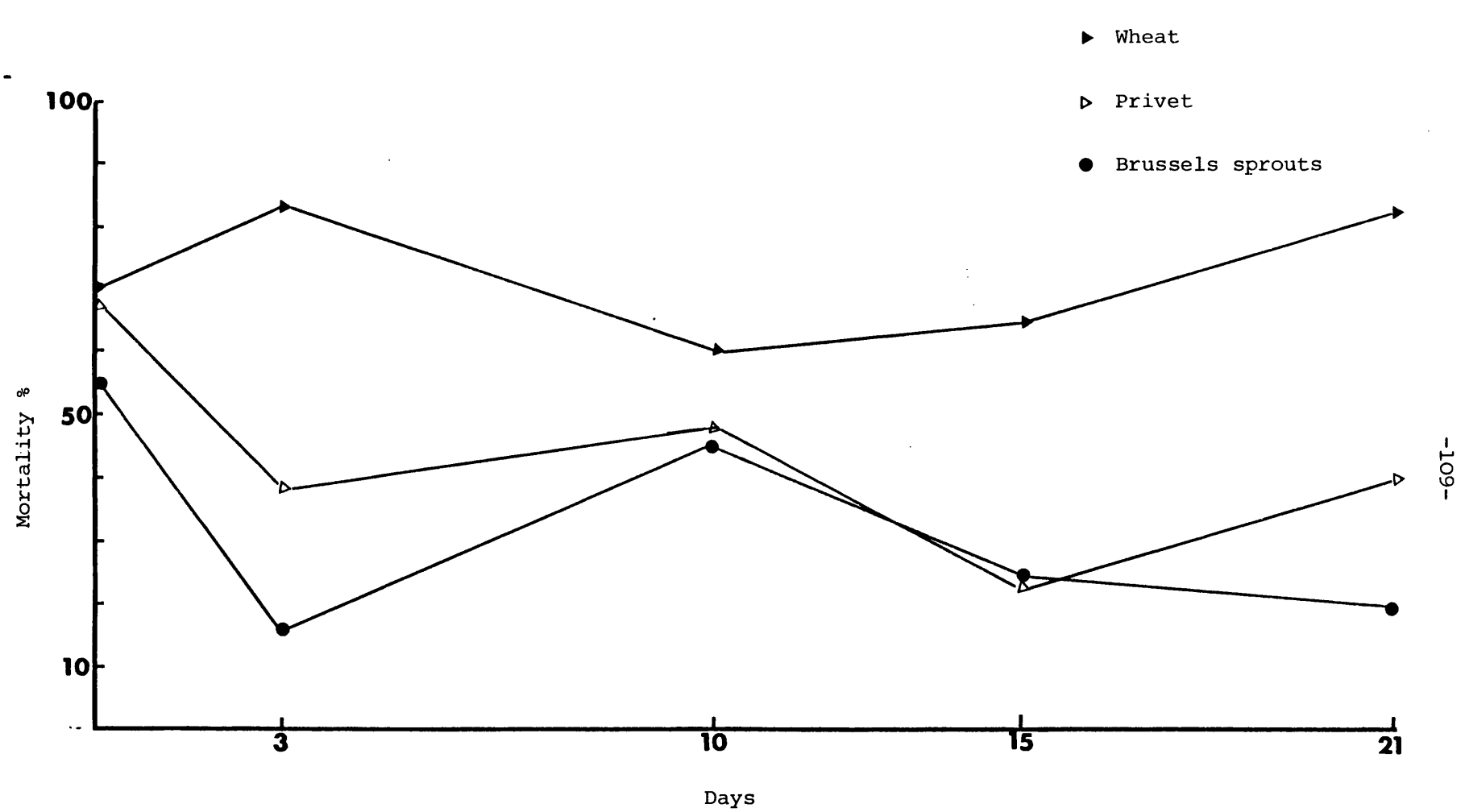

Figure 28. Effects of rainfall on the persistence of permethrin on growing plants.

6.0 General Discussion

Despite the problems of environmental contamination and the development of resistant strains, insecticides are likely to remain for some time as the most powerful weapons in the war against insect pests. They constitute a usually effective, relatively simple and rapid method of pest control. Before new insecticides are marketed they are subjected first to laboratory tests to evaluate their potency and then tested under field conditions. Such assessments can only be determined by utilizing bioassay techniques. These can be designed to evaluate direct contact, residual contact, stomach poison or fumigant effects of the toxicant.

In considering the initial series of experiments, comparing direct contact activity and stomach poison applications with the three insecticides used, the results indicate differences of susceptibility to both bioassay methods and to the insecticides. Irrespective of the method used, permethrin was less effective than either lindane or fenitrothion. This relatively low toxicity of permethrin against locusts, when compared with its high degree of activity against many other insects, has been recorded by other workers (Reay and Ford 1973; Pojananuwong 1976). Both permethrin and fenitrothion were more effective as direct contact poisons, than as stomach poisons, suggesting lower absorbtion through the gut than through the cuticle. In contrast, lindane was more effective as a stomach poison possibly because the exposed topical dose was more subject to volatilization.

In comparing the two methods of offering the stomach poisons to the test insects, the responses varied with each insecticide. With

fenitrothion the differences were negligible, but lindane was more toxic when applied to tissue paper and permethrin more toxic when applied to grass. The reason for these contradictory observations is not apparent, as all the substrates were fed to the locusts within a short time of treatment and eventually all consumed therefore different rates of penetration should not have affected the performance of the toxicants. However, one possible reason could be that tissue paper will pass through the gut intact as it is made up mainly of cellulose and locusts have no cellulase activity (Morgan, 1976). This may be contrasted with grass which will be broken up by both mechanical and enzymic activity. Therefore, it may be that more of the permethrin on grass will be available for absorption than that on the tissue paper. On the basis of this hypothesis one would clearly expect lindane to behave similarly to permethrin. That it does not may perhaps be attributed to the greater volatility of lindane when exposed on grass than on absorbent tissue paper.

The results on the effects of temperature on the persistence of lindane, fenitrothion and permethrin on tissue paper showed that at all temperatures tested, lindane showed least persistence, followed by fenitrothion and permethrin residues retained their toxicities to the locusts throughout the test period. The probable reason for lindane's short persistence is its high volatilic nature (vapour pressure 9.4×10^{-6} mm HG and $20^{o}C$). Ebeling 1963, Gunther 1969, and Quairishi 1977, have given similar reasons for the short persistence of insecticides like lindane. This is further supported by Hartley (1969), who considers that two processes are involved in the vaporisation of an insecticide residue, namely the vapour pressure and the rate of movement away from

the evaporating surface. The importance of volatilization is exemplified by Perry *et al.* (1964) who correlated a considerable difference in loss of topical doses of the closely related organochlorinated insecticides, aldrin and dieldrin, with their different vapour pressures.

Fenitrothion, in contrast to lindane, has a low vapour pressure (6×10^{-6} mm HG at $20^{\circ}C$), which is one of the factors that would account for its moderate persistence. Hattori *et al.* (1976) reported fenitrothion to be relatively stable on wood or mud wall surfaces indoors and Lemon (1967) also showed that fenitrothion was moderately persistent. The degradation of fenitrothion to non-toxic residues is achieved by hydrolysis according to Quraishi (1977).

The results of the persistence of permethrin are in accordance with those of Hadaway *et al.* (1977), who found that under conditions allowing evaporation no loss of permethrin residues could be detected during a 29 day period when used as a residue contact insecticide, against *Glossina austeni*. Whereas Elliot *et al.* (1973), using chemical assay and bioassay of residual contact deposits against *Drosophila melanogaster,* found that 60% of the applied dose remained undecomposed at 20 days. Permethrin's prolonged persistence under the various temperature regimes and with light excluded can be attributed to its low vapour pressure, 3.4×10^{-7} torr at $25^{\circ}C$ (Elliot *et al.*, 1978).

It is known that all the pyrethroids have repellant and antifeedent action. This was confirmed during these studies as it was observed that the locusts took more time to eat the permethrin treated papers than those treated with the other two insecticides.

The comparison of bioassay and colorimetric techniques for the evaluation of fenitrothion residues gave differing results, probably because the chemical method was capable of assaying non-toxic residues. Lindquist *et al.* (1965), using a radio-autographic technique, recorded maximum activity of labelled Bidrin, an organophosphorus systemic insecticide, in plants leaves at a time (14 days after application) when the insecticide was no longer effective against cotton aphids. This type of activity cannot be detected by bioassay techniques but radio or chemical techniques are capable of detecting non- toxic residues especially, as in the present work, the process involved a reaction between nitrobenzyl pyridine and the phosphate components of the insecticide in a slightly basic solution.

Because of the relatively large number of locusts of the same age required for each bioassay, it was not possible to assess persistence at shorter intervals than those used; also, for similar reasons, the experiments were continued for only 21 days. Because of these structures only fenitrothion, with its moderate persistence, shows any substantial increase in degradation at higher temperatures; lindane's persistence was too short and permethrin's too long, even at 30°C, to show any differences.

The effects of light were evaluated at 5°C with a light intensity of 2415.6 lux. This intensity is low compared to light intensity in the tropics, but the spectra, with the lamps used, was similar to natural day light. In the field, especially in the tropics, it is difficult to separate the effects of light from temperatures, because the radiant heat component of light gives an increase in temperature. But in

photochemistry it is the spectrum which is most important, with intensity playing only a secondary role. In comparing the results of the light experiments with the previous ones, involving only temperature as a factor, there is no evidence that light had any additional effect in reducing the persistence of any of the insecticides. In fact, permethrin, for which assessment was continued for 56 days showed a remarkable stability to light. This result substantiates that of Elliot *et al.* (1973) who found that there was no positive effect of light when permethrin treated plywood was kept under a sun lamp and outdoors allowing the UV range of the sun's spectrum to penetrate. They found that the insecticide was persistent for more than 26 days. However, Holmstead *et al.* (1978) found that sunlight has a positive effect on the degradation of permethrin when mixed with a small sample of soil and exposed on a glass plate. The loss of insecticide under sunlight was 55%, but only 35% was lost in total darkness, during a period of 48 days.

The remainder of this experiment involved evaluating persistence on growing plants and since the leaf outside comprises the first and most formidable barrier to the penetration of chemicals into plant tissues, a brief discussion of its origin and composition is considered appropriate.

The cuticle forms the outermost layer of the outer epidermal wall (Figure 29). Priestley (1943) pointed out that most, if not all the tissues of the living plants tend to release fatty substances from living protoplasts. These fatty substances, appear in the walls and if the walls form a continuous system, not directly exposed to air,

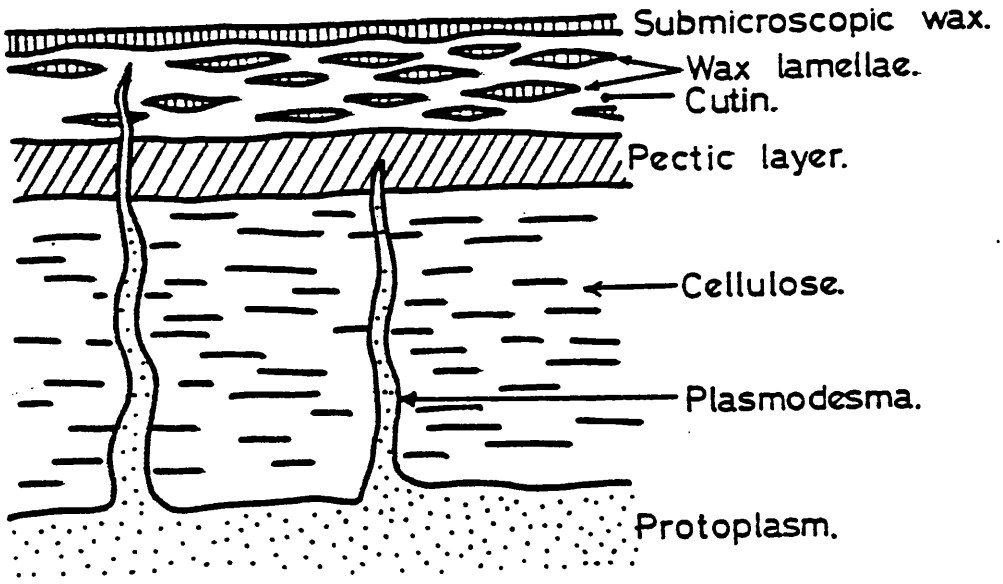

Figure 29. Diagrammatic representation of the outer epidermal cell wall and cuticle of a leaf (from Orgell 1957)

substances may move along to the other regions. However, if such fats are in tissues in which they may have prolonged exposure to air, a cuticle like deposit may form on the outer surfaces of the wall which is in contact with the air. Priestley believed that these fatty substances are esters of fatty acids with higher molecular alcohols and are released into outer epidermal walls, which are saturated with water. Having long hydrocarbon chains without affinity for water, they tend to migrate to the nearest air/water interface, where they accumulate with their hydroxyl or ester group in the water surface and their hydrocarbon chains in the air. The unsaturated linkages among the compound oxidize and gradually link together, with elimination of water from the molecule and a film of more varnish like consistency is gradually formed. It was further demonstrated that surface active substances escape from a normal leaf and diffuse over the surface of the cuticle.

Therefore it is concluded that from young leaves these diffusing substances are mainly lipoids and contribute to the unwettability of the leaf. They become more hydrophilic with increasing age of the leaf, contributing to greater wettability. The differences between the young leaves and old leaves was confirmed by contact angle measurements (Ebeling 1940; Linskens 1950).

In considering the leaf penetration of a chemical, it should be recognised that the surface cuticle continues into the respiratory chambers of the stomata and also into the intercellular spaces of the mesophyll. The entire system of intercellular spaces in leaf, stem, root, flower, and fruit is lined by a suberin lamella, which is defined as a "varnish"-like wall material resistant to strong sulphuric and strong chromic acids (Scott 1950). Internal suberization plays

an important role in the transport of solutes and in permeability, but in contrast to the situation with the outer cuticle, the process of sorption and penetration are modified by the continuous presence of moisture, of particular importance when the penetration of the more polar types of substances is concerned.

The possibilities of the movement of sprayed pesticides from the leaf surface to the phloem and xylem have been described by Currier and Dybing (1959), which is as follows:-

A. Across the cuticle then i) anticlinal walls of epidermis and mesophyll, especially those of the bundle sheath extension or ii) via plasmodesmata in the outer periclinal epidermal wall, and symplast to the phloem or iii) combination of (i) and (ii).

B. Through stomata, then i) across internal cuticle, then wall channels, ii) across internal cuticle, then into plasmodesmata and symplast, iii) in intercellular spaces, spreading along wall surface or in bulk, to vascular bundles or iv) combination of the above.

The morphological and physiological characteristics of a plant are important because they influence the distribution, retention and uptake of the applied insecticide and the effectiveness of the residues. The factors which influence these characteristics have been described by Ebeling (1963) as 1) plant form, e.g. erect, spreading or prostrate, 2) leaf shape, e.g. broad, large, narrow, short or linear, 3) leaf

position and density, e.g. horizontal, upright or pendulus, 4) leaf surface and margin, e.g. hairy or waxy.

Lindane's persistence on growing plants was not evaluated because it had already shown insufficient persistence on inert substrates and excised plant tissues, under the temperature and light regimes used, to warrant further investigation. There was no evidence from the results that the persistence of fenitrothion was affected by any of the plants used, for the LD_{90} persisted for a period between 15 and 21 days, which approximates to the results obtained from the temperature and light experiments. This is not in accord with the results obtained by Miyamoto *et al.* (1965) with Banana, Miyamoto and Sato (1965) with rice and Hosokowa and Miyamoto (1974) with apple; they concluded that fenitrothion is metabolized in situ and the efficacy is retained for only 4 - 5 days. But there is no evidence that these workers evaluated and/or standardised any effects of degradation due to light or temperature.

Although permethrin retained its long persistence (>40 days) on growing plants and the results were variable, there were indications of a greater persistence on wheat than on either Brussels sprouts or privet. Permethrin is known to be readily metabolized by plants, the principal inactivation process involving ester cleavage. Also being lipophilic, it is absorbed readily into the waxy layers on the leaf surface and penetrates the aqueous inner phases, predominantly as more polar metabolites (Elliot *et al.*, 1978). Despite this several workers have reported prolonged persistence on plants and differences in response between plant species. For example, Gaughan and Casida (1978) studied the degradation of ^{14}C labelled permethrin on cotton in

the field and found a 30% loss in the first week, 12% loss in the second week, 7% loss in the third week and 10% loss in 4 - 6 weeks, when the experiment was concluded. These workers also compared the degradation rates on bean and cotton plants under greenhouse conditions and found twice the degradation rate on beans than on cotton. Hadaway *et al.* (1977) evaluated the persistence of permethrin on excised ivy leaves by residual contact bioassay using Tsetse flies; they found that insecticidal activity was still detectable at the end of the experiment, which was of six weeks duration.

The differences in response by wheat, Brussels sprouts or ~~ivy~~ privet in the present work is perhaps contrary to what might have been expected, for penetration of the insecticide into the inner aqueous phase of the leaf would seemingly be facilitated on a leaf surface, like that of wheat, which has a relatively thin layer of wax when compared to Brussels sprouts or privet. But the results were variable and even the nil day assessment gave values considerably in excess of the LD_{50} as determined previously. The only accountable variable was a possible change in response of the test insects. Using the methods detailed in the study it was not practical to adjust the predetermined dose to the weight of individual locusts, as was done orginally with topical applications, so it is possible that the mean weight of locusts used in the tests showed departures from the mean (0.4 g) used. This could have been caused by changes in diet of the locusts in the stock culture necessitated by seasonal shortages. So perhaps a size difference between batches of test insect used was responsible for the variability.

The evaluation of the effects of rainfall was only undertaken with permethrin; of the so called weathering conditions affecting the persistence of insecticides, rainfall is considered to be the most important. But the amount removed or disintegrated through the effects of rainfall is dependent mostly on the chemical characteristics of the insecticide, type of plant surface or other substrate, the length of time involved and the amount of the toxicant originally present. Another important component of rain is the droplet size. It is accepted that large droplets, which have sufficient inertia to resist the angular change in the direction of the stream lines, are not sufficiently diverted from their course and therefore impinge on the surface and are deposited by impact. Equally small droplets travelling at a much higher speed, have sufficient kinetic energy to resist the change in direction, penetrate the boundary layer and are also deposited. Brooks (1947) showed that a dynamic catch exceeding 90% can be expected for droplets larger in diameter (100 µm) at a speed of about 3 m.p.h., but with smaller droplets (40 µm) an air speed of 20 m.p.h. is required to give a similar dynamic catch.

Ripper (1955) has classified rain according to droplet size as follows:

1) Rain drops of 3,000 µm and above as "cloud burst".
2) Rain drops of 2,000 µm as 'heavy rain".
3) Rain drops of 1,000 µm as "modest rain".
4) Rain drops of 500 µm as "light rain".
5) Rain drops of 200-380 µm as "drizzle".

So to assess rain according to water droplet size and to correlate the water quality with the rainfall of Karachi (Pakistan) the water

droplet size was measured by a technique described by Matthews (1975, 1979).

The results of the determination of water droplet size show the nmd (number median diameter) of 3.2 mm (3,200 μm) and vmd (volume median diameter) of 6.2 mm (6,200 μm), see Table 17 and Figure 26. Therefore according to the classification mentioned above, the rain used was "cloud burst". So the simulated rain used was justified from the point of view of rainfall in Karachi (Pakistan), where mean annual rainfall is 7.7 inches, which depends on three months of rainfall during the summer when 20 inches of rain is expected (Wint, 1965).

The effects of simulated rain on the persistence of permethrin was evaluated on wheat, privet and Brussels sprouts. It is known that the pH and temperature of water affects the degradation of insecticides, therefore the pH and temperature of the rain water were measured and found to be 6.8 - 7.0 and 12°C - 15°C respectively. The results of this work indicate that, despite the variability of mortality levels, the effects of rain on the persistence of permethrin, vary according to the substrate. On wheat there is little, if any, loss of permethrin throughout the 21 day period. The loss is greater on privet, especially with the 6 hour rain regime, but most loss occurred on Brussels sprouts, where mortality dropped to 10% and 20% after 21 days. These differences between plants could be attributed to the nature of their leaf surfaces. Both privet and Brussels sprouts leaves have a relatively thick layer of epicuticular wax compared to the leaves of wheat. The permethrin deposit is likely to be more subject to being "washed off" on leaves with a thick wax layer.

Then it is likely that any appreciable lipophilic penetration could have taken place. However, such a postulation, to account for differences in plant response, is dependent on the assumption of rapid absorption into wheat leaves, but, if this occurs, then the insecticide would be at a site where it would be subject to degradation by plant metabolism.

This then raises again the apparent anomaly referred to in the previous experiment, namely, the indication of a longer persistence with wheat, than with Brussels sprouts or privet, because once the rain treatment has had its effect, the remainder of the experimental conditions were similar in the two experiments. Perhaps too much emphasis should not be placed on the effect of plant metabolism in the present series of experiments, because of the relatively low temperatures of 5°C that was used. This was the only temperature that gave any measurable persistence with lindane so, for comparative purposes, its use was continued. At a temperature of 5°C plant metabolism would be slower than at high temperatures and this would presumably affect the rate of degradation of permethrin. This could account for the longer persistence of permethrin when applied to plant surfaces in these experiments when compared to those of other workers. For example, Ohkawa *et al.* (1977) found that the *trans* and *cis* isomers of permethrin, when applied to the leaves of beans, had half lives of 7 and 9 days when the plants were kept at 25°C.

These workers used an analytical technique that separately determined permethrin levels in the surface waxes and within the leaf itself. Their data indicate that loss of permethrin in the surface waxes was approximately as follows:-

Day 0 -- 100%, Day 2 -- 80%, Day 4 -- 60%, Day 8 -- 50%

and Day 14 -- 20%.

This descending order was almost compensated for by an ascending increase in permethrin within the leaf. By using the methods described by these workers, it would be interesting to evaluate the rate of transfer of permethrin from the surface waxes to the inner leaf, using plants with different thickness of wax on their leaves and at different temperature regimes. This would help to resolve the interpretations of some of the results obtained in the present work.

In conclusion these results indicate that, of the three insecticides tested as stomach poisons, fenitrothion is the most effective at equivalent dosage rates. MacCuaig (1974) and Pojananuwag (1976) consider that fenitrothion should continue to be used against locusts as it is effective, costs less than the new pyrethroids and its mammalian toxicity is comparable to that of cismethrin. However, this is not true of permethrin because the geometric mean ratio of toxicity of fenitrothion between insects and mammals is 33 mg/kg, whereas for permethrin it is 4500 mg/kg (Elliot 1977). The major advantages of permethrin as a potential insecticide for locust control, are its low mammalian toxicity and its prolonged persistence. The latter could shift the cost benefit ratio in permethrin's favour, when compared to fenitrothion, by reducing the number of applications . This would be particularly advantageous in controlling locusts in remote areas, where the cost of application, only feasible by using aircraft, outweighs the cost of the insecticide. The accepted safe period for the consumption of crops treated with fenitrothion is 2 - 3 weeks whereas for permethrin no safe period is required. So in situations where it is necessary to spray crops or animal grazing areas then the safeness of permethrin would warrant its usage.

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Appendix 1.

Lindane (2% a.i.) Contact Poison

No. of insect used "n"	Dosage "x"	No. of insect died "r"	Mortality %	P=r/n	q=1-P	P/q	l=lnP/q
10	'C'	-	-	-	-	-	-
10	3	0.5	5	0.05	0.95	0.053	-2.96
10	5	2.0	20	0.20	0.80	0.250	-1.39
10	8	3.0	30	0.30	0.70	0.429	-0.85
10	12	8.0	80	0.80	0.20	4.000	1.39
10	14	9.5	95	0.95	0.05	19.000	2.94

Appendix 2.

W'=Pq	W'x	$W'x^2$	W'l	W'xl
0.048	0.144	0.432	-0.143	-0.427
0.160	0.800	4.000	-0.224	-1.112
0.210	1.680	13.440	-0.178	-1.429
0.160	1.920	23.040	0.224	2.669
0.048	0.672	9.408	0.142	1.976
$\Sigma W=0.626$	$\Sigma W'x=5.216$	$\Sigma W'x^2=50.320$	$\Sigma W'l=-0.179$	$\Sigma Wxl=1.677$

Since n = 10 throughout

$\Sigma'nw' = 6.260$, $\Sigma nW'x = 52.160$, $\Sigma nWx^2 = 503.200$, $\Sigma nW'l = -1.790$,

$\Sigma nWxl = 16.770$

and $\bar{x} = \frac{\Sigma nW'x}{\Sigma nW'} = 8.332$

$\bar{l} = \frac{\Sigma nW'l}{\Sigma nW'} = -0.029$

$\hat{\beta} = \frac{\Sigma nW'\Sigma nW'xl - \Sigma nW'x\ \Sigma nW'l}{\Sigma nW'\Sigma nW'x^2 - (nW'x)^2} = 0.462$

$\hat{\alpha} = \bar{l} - \hat{\beta}\bar{x} = -3.878$

$C = -\frac{\hat{\alpha}}{\hat{\beta}} = 8.391$

Standard Errors for

$$\delta^2(\hat{\alpha}) = \frac{1}{\{ nW' \}} + \frac{\bar{x}^2}{n\{\Sigma W'x^2 - \frac{(\Sigma W'x)^2}{\Sigma W}}$$

$$= 1.172$$

$$\delta^2(\hat{\alpha}^{-1}) = \frac{1}{n\{\Sigma W' \}} = 0.160$$

$$\delta^2(\hat{\beta}) = \frac{1}{n\{\Sigma W'(x-\bar{x})^2\}}$$

$$= \frac{1}{n\ \Sigma W'x^2 - \frac{(W'x)^2}{\Sigma W'}}$$

$$= 0.015$$

$$\delta^2(C) = 1/\hat{\beta}^2 \{ \delta^2(\hat{\alpha}^{-1}) + (C-\bar{x})^2 \times \delta^2(\hat{\beta}) \}$$

$$= 0.749$$

Confidence Intervals for

$$\hat{\alpha} = \hat{\alpha} \pm t_\alpha \sqrt{\delta^2(\hat{\alpha})}$$

$$= -3.877 \quad 1.96 \sqrt{1.172}$$

$$-1.75, -5.99$$

$$\hat{\beta} = \hat{\beta} \pm t_\alpha \sqrt{\delta^2(\hat{\beta})}$$

$$= 0.462 \pm 1.96 \sqrt{0.015}$$

$$+0.22, 0.70$$

$$C = C \pm t_\alpha \sqrt{\delta^2(C)}$$

$$= 8.39 \pm 1.96 \quad 0.749$$

$$6.69, 10.08$$

$$LD_{50} = \frac{\ln 1 - \hat{\alpha}}{\hat{\beta}} = 8.391 \text{ µg/gm}$$

$$LD_{80} = \frac{\ln 4 - \hat{\alpha}}{\hat{\beta}} = 11.394 \text{ µg/gm}$$

$$LD_{90} = \frac{\ln 9 - \hat{\alpha}}{\hat{\beta}} = 13.149 \text{ µg/gm}$$

Standard errors for

$$LD_{50} = 1/\hat{\beta}^2 \{\delta^2(\hat{\alpha}^1) + (LD_{50} - \bar{x})^2 \times \delta^2(\hat{\beta})\}$$
$$= 0.749$$

$$LD_{80} = 1/\hat{\beta} \{\delta^2(\hat{\alpha}^1) + (LD_{80} - \bar{x})^2 \times \delta^2(\hat{\beta})\}$$
$$= 1.408$$

$$LD_{90} = 1/\hat{\beta} \{\delta^2(\hat{\alpha}^1) + (LD_{90} - \bar{x})^2 \times \delta^2(\hat{\beta})\}$$
$$= 2.380$$

Confidence intervals for

$$LD_{50} = LD_{50} \pm t_\alpha \sqrt{\delta^2(LD_{50})}$$
$$= 8.391 \pm 1.96 \sqrt{0.75}$$

6.69, 10.09

$$LD_{80} = LD_{80} \pm t_\alpha \sqrt{\delta^2(LD_{80})}$$
$$= 11.391 \pm 1.96 \sqrt{1.408}$$

9.06, 13.71

$$LD_{90} = LD_{90} \pm t_\alpha \sqrt{\delta^2(LD_{90})}$$
$$= 13.14 \pm 1.96 \sqrt{2.380}$$

10.12, 16.17

Appendix 3.

Logit χ^2

x	W'	l	l^	$(l-l^)^2$	$W'(l-l^)^2$
3	0.048	-2.960	-2.491	0.220	0.0105
5	0.160	-1.390	-1.567	0.031	0.0050
8	0.210	-0.850	-0.181	0.447	0.0939
12	0.160	1.390	1.667	0.076	0.0122
14	0.048	2.940	2.591	0.121	0.0058
					0.1274

$$\chi^2_3 = n \{\Sigma W'(l-l^)^2\}$$

$$= 1.27 \text{ NS } P < 0.05$$

The value of $\chi_3^2 = 1.27$, which is not significant as significance at 5% level $\chi_3^2 > 7,82$.

Appendix 4.

Logistic Curve $\frac{1}{1+e^{-(\alpha\hat{}+\beta\hat{}x)}} = \frac{1}{1+e^{-1\hat{}}} = P$

$-1\hat{}$	$e^{-1\hat{}}$	$1+e^{-1\hat{}}$	$\frac{1}{1+e^{-1\hat{}}}$	y = 10xP
3.415	30.416	31.416	0.0318	0.318
2.953	19.163	20.163	0.0495	0.495
2.491	12.073	13.073	0.0764	0.764
2.029	7.606	8.606	0.1161	0.161
1.567	4.792	5.792	0.1726	1.726
1.105	3.092	4.092	0.2488	2.488
0.643	1.902	2.902	0.3445	3.445
0.181	1.198	2.198	0.4548	4.548
-0.281	0.755	1.755	0.5697	5.697
-0.743	0.475	1.475	0.6776	6.776
-1.205	0.299	1.299	0.7694	7.694
-1.667	0.188	1.188	0.8411	8.411
-2.129	0.118	1.118	0.8936	8.936
-2.591	0.074	1.074	0.9302	9.302

Appendix 5.

Lindane (2% a.i.) Stomach Poison/Grass

"n"	"x"	No. of insect died "r"	Mortality %	P=r/n	q=1-P	P/q	l=ln P/q
10	"C"	-	-	-	-	-	-
10	3	0.5	5	0.05	0.95	0.052	-2.956
10	5	1	10	0.10	0.90	0.11	-2.198
10	8	6	60	0.60	0.40	1.50	0.405
10	12	9	90	0.90	0.10	9.00	2.197
10	14	9.5	95	0.95	0.05	19.00	2.944

Appendix 6.

W'=Pq	W'x	Wx^2	W'l	W'xl
0.0475	0.1425	0.4275	-0.1404	-0.4212
0.0900	0.4500	2.2500	-0.1978	-0.9891
0.2400	1.9200	15.3600	0.0972	0.7776
0.0900	1.0800	12.9600	0.1977	2.3727
0.0475	0.6650	9.3100	0.1398	1.9577
0.5150	4.2575	40.3075	0.0965	3.6977

Since n = 10

$nW' = 5.150$ $nW'x = 42.575$ $nW\bar{x}^2 = 403.075$

$nW'l = 0.965$ $nW'xl = 36.977$

$$\bar{x} = \frac{\Sigma nW'x}{\Sigma nW'} = 8.266$$

$$\bar{l} = \frac{\Sigma nW'l}{\Sigma nW'} = 0.187$$

$$\hat{\beta} = \frac{\Sigma nW' \Sigma nW'xl - nW'x\ nW'l}{\Sigma nW' \Sigma nW'x^2 - (\Sigma nW'x)^2} = 0.567$$

$$\hat{\alpha} = \bar{l} - \hat{\beta}\bar{x} = -4.50$$

$$C = -\frac{\hat{\alpha}}{\hat{\beta}} = 7.93$$

Standard Errors and Confidence Intervals for

$$\delta^2(\hat{\alpha}) = \frac{1}{\{nW'\}} + \frac{\bar{x}^2}{n\{W'x^2-\frac{(W'x)^2}{W'}\}} = 1.5310$$

$$\delta^2(\hat{\alpha}^1) = \frac{1}{n\{W'\}} = 0.1942$$

$$\delta^2(\hat{\beta}) = \frac{1}{n\{W'(x-\bar{x})^2\}}$$

$$= \frac{1}{n\{W'x^2-\frac{(W'x)^2}{W'}\}} = 0.0196$$

$$\delta^2(C) = 1/(\hat{\beta})^2\{\delta^2(\hat{\alpha}^1) + (C-\bar{x})^2 \times \delta^2(\hat{\beta}) = 0.61$$

C.I. $\hat{\alpha}$ $= \hat{\alpha} \pm t_\alpha \sqrt{\delta^2(\hat{\alpha})}$

$= -4.50 \pm 1.96 \sqrt{1.5310}$

$-2.07, -6.92$

$\hat{\beta}$ $= \hat{\beta} \pm t_\alpha \sqrt{\delta^2(\hat{\beta})}$

$= 0.567 \pm 1.96 \sqrt{0.0196}$

$0.29, 0.84$

$C = C \pm t_\alpha \sqrt{\delta^2(C)}$

$= 7.93 \pm 1.96 \sqrt{0.61}$

$6.40, 9.46$

Lethal Doses

$$LD_{50} = \frac{\ln 1-\hat{\alpha}}{\hat{\beta}} = 7.93$$

$$LD_{80} = \frac{\ln 4-\hat{\alpha}}{\hat{\beta}} = 10.38$$

$$LD_{90} = \frac{\ln 9-\hat{\alpha}}{\hat{\beta}} = 11.81$$

Standard Errors for

$$LD_{50} = 1/\beta\hat{}^2 \{ \delta^2(\alpha\hat{}^{-1}) + (LD_{50}-\bar{x})^2 \times \delta^2(\beta\hat{}) \}$$

$$= 0.61$$

$$LD_{80} = 1/\beta\hat{}^2 \{\delta^2(\alpha\hat{}^{-1}) + (LD_{80}-\bar{x})^2 \times \delta^2(\beta\hat{}) \}$$

$$= 0.88$$

$$LD_{90} = 1/(\beta\hat{})^2 \{\delta^2(\alpha\hat{}^{-1}) + (LD_{90}-\bar{x})^2 \times \delta^2(\beta\hat{}) \}$$

$$= 1.37$$

Confidence intervals for

$$LD_{50} = LD_{50} \pm t_\alpha \sqrt{\delta^2(LD_{50})}$$

$$= 7.93 \pm 1.96 \sqrt{0.61}$$

6.40, 9.46

$$LD_{80} = LD_{80} \pm t_\alpha \sqrt{\delta^2(LD_{80})}$$

$$= 10.38 \pm 1.96 \sqrt{0.88}$$

8.54, 12.21

$$LD_{90} = LD_{90} \pm t_\alpha \sqrt{\delta^2(LD_{90})}$$

$$= 11.81 \pm 1.96 \sqrt{1.37}$$

9.51, 14.10

Appendix 7.

Logit χ^2

x	W'	l	l^	$(l-l^)^2$	$W'(l-l^)^2$
3	0.0475	-2.956	-2.980	0.0005	0.0274
5	0.0900	-2.198	-1.977	0.0488	0.0044
8	0.2400	0.405	0.027	0.1428	0.0342
12	0.0900	2.197	2.433	0.0557	0.0050
14	0.0475	2.944	3.635	0.4774	0.0226
					0.09368

$$\chi^2_3 = n\{W'(l-l^)^2\}$$

$$= 0.9368 \quad P < 0.05$$

The values for χ^2 is not significant, as the significance at 5% level the $\chi^2_3 > 7.82$

Appendix 8.

Logistic Curve

$$\frac{1}{1+e^{-(\alpha\hat{}+\beta\hat{}x)}} = \frac{1}{1+e^{-1\hat{}}} = P$$

$-1\hat{}$	$e^{-1\hat{}}$	$1+e^{-1\hat{}}$	$\frac{1}{1+e^{-1\hat{}}}$	y=10xP
4.183	65.5622	66.5622	0.01502	0.1502
3.581	35.9094	36.9094	0.02709	0.2709
2.980	19.6878	20.6878	0.04833	0.4833
2.378	10.7833	11.7833	0.08486	0.8486
1.777	5.9120	6.9120	0.14467	1.4467
1.170	3.2219	4.2219	0.23685	2.3685
0.574	1.7753	2.7753	0.36031	3.6031
-0.027	0.9733	1.9733	0.50674	5.0674
-0.628	0.5336	1.5336	0.65203	6.5203
-1.230	0.2922	1.2922	0.77381	7.7381
-1.830	0.1602	1.1602	0.86188	8.6188
-2.433	0.0877	1.0877	0.91930	9.1930
-3.035	0.0480	1.0480	0.95413	9.5413
-3.635	0.0263	1.0263	0.97429	9.7429
-4.238	0.0144	1.0144	0.98577	9.8577
-4.839	0.0079	1.0079	0.99215	9.9215
-5.441	0.0043	1.0043	0.99568	9.9568

Appendix 9.

Lindane (2% a.i.) Stomach Poison 'Tissue Paper'

"n"	"x"	No. of insect died "r"	Mortality %	P=r/n	q=1-P	P/q	l=ln P/q
10	"C"	-	-	-	-	-	-
10	2	0.5	5	0.05	0.95	0.0526	-2.9450
10	4	2.0	20	0.20	0.80	0.2500	-1.3862
10	6	8.0	80	0.80	0.20	0.4000	1.3862
10	8	9.0	90	0.90	0.10	9.0000	2.1972
10	10	9.5	95	0.95	0.05	19.0000	2.9444

Appendix 10.

W'=Pq	W'x	$W'x^2$	W'l	W'xl
0.0475	0.095	0.1900	-0.13988	-0.27977
0.1600	0.640	2.5600	-0.22179	-0.88716
0.1600	0.960	5.7600	0.22179	1.33075
0.0900	0.720	5.7600	0.19774	1.58918
0.0475	0.475	4.7500	0.13985	1.39859
0.5050	2.8900	19.0200	0.19771	3.14439

Since n = 10

nW' = 5.05 nWx = 28.90 $nW'x^2$ = 190.20 nW'l = 1.97

nW'xl = 31.43

$$\bar{x} = \frac{\Sigma nW'x}{\Sigma nW'} = 5.722$$

$$\bar{l} = \frac{\Sigma nW'l}{\Sigma nW'} = 0.390$$

$$\hat{\beta} = \frac{\Sigma nW' \Sigma nW'xl - \Sigma nW'x \; \Sigma nW'l}{\Sigma nW' \Sigma nW'x^2 - \{(nW'x)^2\}} = 0.812$$

$$\hat{\alpha} = 1 - \hat{\beta}\,\bar{x} = -4.256$$

$$C = -\frac{\hat{\alpha}}{\hat{\beta}} = 5.24$$

Standard errors and confidence intervals

$$\delta^2(\hat{\alpha}) = \frac{1}{nW'} + \frac{\bar{x}^2}{n\,W'x^2 - \frac{(w'x)^2}{W'}} = 1.518$$

$$\delta^2(\hat{\alpha}^{-1}) = \frac{1}{n\{W'\}} = 0.198$$

$$\delta^2(\hat{\beta}) = \frac{1}{n\{W'x^2 - (x-\bar{x})^2}$$

$$= \frac{1}{n\{W'x^2 - \frac{(W'x)^2}{W}} = 0.040$$

$$\delta^2(C) = 1/(\hat{\beta})^2 \{\delta^2(\hat{\alpha}^{-1}) + (C-\bar{x})^2 \times \delta^2(\hat{\beta}) = 0.31$$

Confidence Intervals

C.I. for $\hat{\alpha}$ $= \hat{\alpha} \pm t_\alpha \sqrt{\delta^2(\hat{\alpha})}$

$= -4.256 \pm 1.96 \sqrt{1.518}$

$-1.84, -6.67$

C.I. for $\hat{\beta}$ $= \hat{\beta} \pm t_\alpha \sqrt{\delta^2(\hat{\beta})}$

$= 0.812 \pm 1.96 \sqrt{0.040}$

$0.42, 1.20$

C.I. for C $= C \pm t_\alpha \sqrt{\delta^2(C)}$

$= 5.24 \pm 1.96 \sqrt{0.31}$

$4.15, 6.33$

Lethal Doses

$$LD_{50} = \frac{\ln 1 - \hat{\alpha}}{\hat{\beta}} = 5.24$$

$$LD_{80} = \frac{\ln 4 - \hat{\alpha}}{\hat{\beta}} = 6.94$$

$$LD_{90} = \frac{\ln 9 - \hat{\alpha}}{\hat{\beta}} = 7.95$$

Standard Errors for

$$LD_{50} = LD_{50} \pm t_\alpha \sqrt{\delta^2(LD_{50})}$$

4.15, 6.33

$$LD_{80} = LD_{80} \pm t_{\alpha} \sqrt{\delta^2 (LD_{80})}$$

$$= 6.94 \pm 1.96 \sqrt{0.40}$$

5.70, 8.18

$$LD_{90} = LD_{90} \pm t_{\alpha} \sqrt{\delta^2 (LD_{90})}$$

$$= 7.95 \pm 1.96 \sqrt{0.60}$$

6.42, 9.46

Appendix 11

Logit χ^2

X	W'	l	l^	$(l-l^)^2$	$W'(l-l^)^2$
2	0.0475	-2.9450	-2.631	0.0986	0.0046
4	0.1600	-1.3862	-1.007	0.1438	0.0230
6	0.1600	1.3862	0.616	0.5932	0.0949
8	0.0900	2.1972	2.240	0.0018	0.0001
10	0.0475	2.9444	3.865	0.8475	0.0402
					0.1628

$$\chi^2_3 = n\{ W'(l-l^)^2 \}$$

$$= 1.628 \text{ N.S.} \qquad P < 0.05$$

χ^2_3 = 1.62 which is not significant, as the significance at 5% level the $\chi^2_3 > 7.82$.

Appendix 12

Logistic Curve

$$\frac{1}{1+e^{-(\alpha\hat{}+\beta\hat{}x)}} = \frac{1}{1+e^{-1\hat{}}} = P$$

$-1\hat{}$	$e^{-1\hat{}}$	$1+e^{-1\hat{}}$	$\frac{1}{1+e^{-1\hat{}}}$	Y=10xP
3.443	31.280	32.280	0.0309	0.309
2.631	13.887	14.887	0.0671	0.67
1.819	6.165	7.165	0.1395	1.395
1.007	1.215	3.737	0.2675	2.675
0.195	1.215	1.215	0.4514	4.514
-0.616	0.540	1.540	0.6493	6.493
-1.428	0.239	1.239	0.8065	8.065
-2.240	0.106	1.106	0.9037	9.037
-3.052	0.047	1.047	0.9548	9.548
-3.865	0.020	1.020	0.9794	9.794
-4.677	0.009	1.009	0.9907	9.707
-5.489	0.004	1.004	0.9958	9.958
-6.301	0.001	1.001	0.9981	9.981
-7.113	0.0008	1.0008	0.9991	9.991

Appendix 13

Fenitrothion (2% a.i.) Contact Poison

"n"	"x"	"r"	Mortality %	P=r/n	q=1-P	P/q	l=lnP/q
20	"c"	-	-	-	-	-	-
20	2	0.1	5	0.05	0.95	0.052	-2.944
20	4	6.0	30	0.30	0.70	0.428	-0.847
20	6	14.0	70	0.70	0.30	2.333	0.847
20	8	18.0	90	0.90	0.10	9.000	2.197
20	10	19.5	95	0.95	0.05	19.00	2.944

Appendix 14

Fenitrothion (2% a.i.) Contact Poison

W'=Pq	W'x	$W'x^2$	W'l	W'Xl
0.0475	0.0950	0.1900	-0.1398	-0.2796
0.2100	0.8400	3.3600	-0.1778	-0.7114
0.2100	1.2600	7.5600	0.1778	1.0672
0.0900	0.7200	5.7600	0.1977	1.5818
0.0475	0.4750	4.7500	0.1398	1.3985
0.6050	3.3900	21.6200	0.1977	3.0565

Since n = 20

$nW' = 12.10$, $nW'x = 67.80$, $nW'x^2 = 432.40$, $nW'l = 3.954$, $nW'xl = 61.13$

$$\bar{x} = \frac{\Sigma nW'x}{\Sigma nW'} = 5.60$$

$$\bar{l} = \frac{\Sigma nW'l}{\Sigma nW'} = 0.326$$

$$= \frac{\Sigma nW' \times \Sigma nW'xl - \Sigma nW'x \times nW'l}{\Sigma nW' \times \Sigma nW'x^2 - \Sigma(nW'x)^2} = 0.742$$

$$\alpha\hat{} = \bar{l} - \beta\hat{}\bar{x} = -3.829$$

$$C = \frac{-\alpha\hat{}}{\beta\hat{}} = 5.160$$

Standard errors for

$$\delta^2(\hat{\alpha}) = \frac{1}{nW'} + \frac{\bar{x}^2}{n\,W'x^2 - \frac{(W'x)^2}{W'}} = 0.68$$

$$\delta^2(\alpha\hat{}') = \frac{1}{n\{W'\}} = 0.083$$

$$\delta^2(\beta\hat{}) = \frac{1}{n\,W'x^2 - \frac{(W'x)^2}{W}} = 0.019$$

$$\delta^2(C) = 1/(\beta\hat{})^2\{\delta^2(\alpha\hat{}') + (C-\bar{x})^2 \times \delta^2(\beta\hat{})\} = 0.157$$

Confidence intervals for

$$\alpha\hat{} = \alpha\hat{} \pm t_\alpha \sqrt{\delta^2(\alpha\hat{})}$$

$$= -3.83 \pm 1.96 \sqrt{0.68} = -2.21, -5.44$$

$$\beta\hat{} = \beta\hat{} \pm t_\alpha \sqrt{\delta^2(\beta\hat{})}$$

$$= 0.742 \pm 1.96 \sqrt{0.019} = 0.47, 1.01$$

$$C = C \pm t_\alpha \sqrt{\delta^2(C)}$$

$$= 5.16 \pm 1.96 \sqrt{0.157} = 4.38, 5.94$$

Lethal doses

$$LD_{50} = \frac{\ln 1-\alpha\hat{}}{\beta\hat{}} = 5.16$$

$$LD_{80} = \frac{\ln 4 -\alpha\hat{}}{\beta\hat{}} = 7.03$$

$$LD_{90} = \frac{\ln 9 - \alpha\hat{}}{\beta\hat{}} = 8.12$$

Standard errors for

$$LD_{50} = 1/(\beta\hat{})^2\{\delta^2(\alpha\hat{}') + (LD_{50} - \bar{x})^2 \times \delta^2(\beta\hat{})\} = 0.157$$

$$LD_{80} = 1/(\beta\hat{})^2\{\delta^2(\alpha\hat{}') + (LD_{80} - \bar{x})^2 \times \delta^2(\beta\hat{})\} = 0.221$$

$$LD_{90} = 1/(\beta\hat{})^2\{\delta^2(\alpha\hat{}) + (LD_{90} - \bar{x})^2 \times \delta^2(\beta\hat{})\} = 0.369$$

Confidence intervals for

$$LD_{50} = LD_{50} \pm t_\alpha \sqrt{\delta^2(LD_{50})}$$

$$= 5.16 \pm 1.96 \sqrt{0.157} = 4.38, 5.94$$

$$LD_{80} = LD_{80} \pm t_{\alpha} \sqrt{\delta^2(LD_{80})}$$

$$= 7.03 \pm 1.96 \sqrt{0.221} = 6.10, 7.95$$

$$LD_{90} = LD_{90} \pm t_{\alpha} \sqrt{\delta^2(LD_{90})}$$

$$= 8.12 \pm 1.96 \sqrt{0.369} = 6.93, 9.31$$

Appendix 15

Logit χ^2

x	W'	l	l^	$(l-l^)^2$	$W'(l-l^)^2$
2	0.0475	-2.944	-3.784	0.7056	0.0335
4	0.2100	-0.9847	-1.544	0.4858	0.1020
6	0.2100	0.847	0.696	0.0228	0.0047
8	0.0900	2.197	2.936	0.5461	0.0491
10	0.0475	2.944	5.176	4.9818	0.2366
					0.4259

$$\chi^2_3 = n\{W' (l-l^)^2\} = 8.518 \quad S$$

$P < 0.05$

The value of $\chi^2_3 = 8.518$, which is significant, as the significance at 5% level, the $\chi^2_3 > 7.82$

Appendix 16

Logistic curve $\frac{1}{1+e^{-(\hat{\alpha}+\hat{\beta}\hat{x})}} = \frac{1}{1+e^{-\hat{l}}} = P$

$-\hat{l}$	$e^{-\hat{l}}$	$1+e^{-\hat{l}}$	$\frac{1}{1+e^{-\hat{l}}}$	Y=20xP
4.904	134.828	135.828	0.0074	0.1474
3.784	43.9916	44.9916	0.0222	0.4445
2.664	14.3535	15.3535	0.0651	1.3026
1.554	4.6832	5.6832	0.3955	7.9119
0.424	1.5280	2.5280	0.6673	13.3460
-0.696	0.4985	1.4985	0.6673	17.2017
-1.816	0.1626	1.1626	0.8600	17.2017
-2.936	0.0530	1.0530	0.9459	18.9919
-4.055	0.0173	1.0173	0.9829	19.6591
-5.176	0.0056	1.0056	0.9943	19.8876

Appendix 17

Fenitrothion (2% a.i.) Stomach poison/Grass

"n"	"x"	"r"	Mortality %	P=r/n	q=1-P	P/q	l=lnP/q
10	"C"	-	-	-	-	-	-
10	2	0.5	5	0.05	0.95	0.05263	-2.944
10	4	2.0	20	0.20	0.80	0.2500	-1.386
10	6	3.0	30	0.30	0.70	0.4285	-0.847
10	8	6.0	60	0.60	0.40	1.5000	0.4054
10	10	8.0	80	0.80	0.20	4.000	1.3862
10	12	9.0	90	0.90	0.10	9.000	2.1972
10	14	9.0	90	0.90	0.10	9.000	2.1972
10	16	9.5	95	0.95	0.05	19.000	2.9444

Appendix 18

W'=Pq	W'x	$W'x^2$	W'l	W'xl
0.0475	0.0950	0.1900	-0.1398	-0.2797
0.1600	0.6400	2.5600	-0.2218	-0.8870
0.2100	1.2600	7.5600	0.1778	-1.0672
0.2400	1.9200	15.3600	0.0972	0.7783
0.1600	1.6000	16.000	0.2217	2.2179
0.0900	1.0800	12.9600	0.1977	2.3729
0.0900	1.2600	17.6400	0.1977	2.7684
0.0475	0.7600	12.1600	0.1398	2.2377
1.0450	8.6150	84.4300	0.3149	8.1413

Since n = 10

$NW' = 10.450$, $nW'x = 86.150$, $nW'x^2 = 844.300$, $nW'l = 3.149$, $nW'xl = 81.413$

$$\bar{x} = \frac{nW'x}{nW'} = 8.24$$

$$\bar{l} = \frac{nW'l}{nW'} = 0.30$$

$$\hat{\beta} = \frac{\Sigma nW' \times \Sigma nW'l - \Sigma nW'x \, \Sigma nW'l}{\Sigma nW' \times nW'x^2 - \{ (nW'x)^2 \}} = 0.413$$

$$\hat{\alpha} = \bar{l} - \hat{\beta}\bar{x} = -3.102$$

$$C = \frac{-\hat{\alpha}}{\hat{\beta}} = 7.510$$

Standard errors for

$$\delta^2(\hat{\alpha}) = \frac{1}{nW'} + \frac{\bar{x}}{n\{W'x^2 - \frac{(W'x)^2}{W'}\}} = 0.602$$

$$\delta^2(\hat{\alpha}') = \frac{1}{n\{W'\}} = 0.095$$

$$\delta^2(\hat{\beta}) = \frac{1}{n\{W'x^2 - \frac{(W'x)^2}{W'}\}} = 0.007$$

$$\delta^2(C) = 1/\hat{\beta}\ \{\delta^2(\hat{\alpha}) + (C-\bar{x})^2 \times \delta(\hat{\beta})\} = 0.583$$

Confidence intervals for

$$\hat{\alpha} = \hat{\alpha} \pm t_\alpha \sqrt{\delta^2(\hat{\alpha})}$$

$$= -3.102 \pm 1.96\sqrt{0.602} = 1.58, -4.62$$

$$\hat{\beta} = \beta\cdot \pm t_\alpha \sqrt{\delta^2(\hat{\beta})}$$

$$= 0.413 \pm 1.96\ \sqrt{0.007} \qquad 0.24, 0.58$$

$$C = C \pm t_\alpha \sqrt{\delta^2(C)}$$

$$= 7.50 \pm 1.96\ \sqrt{0.583} \qquad 6.00, 8.99$$

Lethal doses

$$LD_{50} = \frac{\ln 1 - \hat{\alpha}}{\hat{\beta}} = 7.510$$

$$LD_{80} = \frac{\ln 4 - \hat{\alpha}}{\hat{\beta}} = 10.867$$

$$LD_{90} = \frac{\ln 9 - \hat{\alpha}}{\hat{\beta}} = 12.831$$

Standard errors for

$$LD_{50} = 1/(\hat{\beta})^2\{\delta^2(\hat{\alpha}) + (LD_{50} - \bar{x})^2 \times \delta^2(\hat{\beta})\} = 0.583$$

$$LD_{80} = 1/(\hat{\beta})^2\{\delta^2(\hat{\alpha}') + (LD_{50} - \bar{x})^2 \times \delta^2(\hat{\beta})\} = 0.840$$

$$LD_{90} = 1/(\hat{\beta})^2\{\delta^2(\hat{\alpha}) + (LD_{90} - \bar{x})^2 \times \delta^2(\hat{\beta})\} = 1.421$$

Confidence intervals for

$LD_{50} = LD_{50} \pm t_{\alpha} \sqrt{\delta^2(LD_{50})}$

$7.510 \pm 1.96 \sqrt{0.583}$ 6.00, 8.990

$LD_{80} = LD_{80} \pm t_{\alpha} \sqrt{\delta^2(LD_{80})}$

$10.867 \pm 1.96 \sqrt{0.840}$ 9.070, 12.663

$LD_{90} = LD_{90} \pm t_{\alpha} \sqrt{\delta^2(LD_{90})}$

$12.831 \pm 1.96 \sqrt{1.421}$ 10.494, 15.167

Appendix 19

Logit χ^2

x	W'	l	l^	$(l-l^)^2$	$W'(l-l^)^2$
2	0.0475	-2.944	-2.275	0.4475	0.2125
4	0.1600	-1.386	-1.448	0.0038	0.0006
6	0.2100	-0.847	-0.621	0.0510	0.0172
8	0.2400	0.405	0.205	0.0400	0.0096
10	0.1600	1.386	1.032	0.1253	0.0200
12	0.0900	2.197	1.859	0.1142	0.0102
14	0.0900	2.197	2.686	0.2391	0.0215
16	0.0475	2.944	3.514	0.3237	0.0153

$$\chi^2_6 = n\{W'(l-l^)^2\} = 3.069 \text{ NS } P < 0.05$$

The value of $\chi^2_6 = 3.069$, which is not significant as the significance at 5% level, the $\chi^2_6 > 12.59$

Appendix 20

Logistic Curve $\frac{1}{1+e^{-(\alpha\hat{}+\beta\hat{}x)}} = \frac{1}{1+e^{-1\hat{}}} = P$

-1	e^{-1}	$1+e^{-1}$	$\frac{1}{1+e^{-1}}$	Y=10xP
2.688	14.7022	15.7022	0.063685	0.63685
2.275	9.7279	10.7279	0.093214	0.93214
1.861	6.4301	7.4301	0.134586	1.34586
1.448	4.2555	5.2555	0.190278	1.90278
1.634	2.8142	3.8142	0.262137	2.62173
0.621	1.8611	2.8611	0.349508	3.4950
0.2077	1.2308	2.2308	0.448260	4.4826
-0.2058	0.81399	1.81399	0.5512691	5.512691
-0.6193	0.53832	1.53832	0.650059	6.50059
-1.0328	0.35600	1.35600	0.737458	7.37458
-1.4463	0.23543	1.23543	0.809428	8.09428
-1.8598	0.15570	1.15570	0.865273	8.65273
-2.2733	0.10297	1.10297	0.906641	9.06641
-2.6868	0.068098	1.068098	0.936242	9.3624
-3.1003	0.045035	1.045035	0.956905	9.56905
-3.5138	0.029783	1.029783	0.971077	9.71077

Appendix 21

Fenitrothion (2% a.i.). Stomach poison/Tissue paper

"n"	"x"	"r"	Mortality %	P=r/n	q=1-P	P/q	l=lnP/q
20	"C"	-	-	-	-	-	-
20	2	3	15	0.15	0.85	0.1764	-1.7346
20	4	5	25	0.25	0.75	0.3333	-1.0986
20	6	7	35	0.35	0.65	0.5384	-0.6190
20	8	12	60	0.60	0.40	1.5000	0.4054
20	10	14	70	0.70	0.30	2.3333	0.8472
20	12	19.5	97.5	0.975	0.025	39.000	3.6635

Appendix 22

W'=Pq	W'x	$W'x^2$	W'l	W'xl
0.1275	0.2550	0.5100	-0.2211	-0.4423
0.1875	0.7500	3.0000	-0.2059	-0.8239
0.2275	1.3650	8.1900	-0.1408	-0.8449
0.2400	1.9200	15.3600	0.0972	0.7783
0.2100	2.1000	21.0000	0.1779	1.7791
0.0243	0.2920	3.5100	0.0893	1.0715
1.0168	6.6820	51.5700	-0.2034	1.5178

Since n = 20

nW' = 20.336, nW'x = 133.64, $nW'x^2$ = 1031.400, nW'l = -4.069,

nW'xl = 30.357

$$\bar{x} = \frac{nW'x}{nW'} = 6.657$$

$$\bar{l} = \frac{nW'l}{nW'} = -0.2000$$

$$\hat{\beta} = \frac{\Sigma nW'x \Sigma nW'xl - \Sigma nW'x \Sigma nW'l}{\Sigma nW' \times \Sigma nW'x^2 - \{(nW'x)^2\}} = 0.37$$

$$\hat{\alpha} = \hat{l} - \hat{\beta}\bar{x} = -2.65$$

$$C = \frac{\hat{\alpha}}{\hat{\beta}} = 7.16$$

Standard Errors

$$\delta^2(\hat{\alpha}) = \frac{1}{\Sigma nW'} + \frac{\bar{x}^2}{n\left\{W'x^2 - \frac{(W'x)^2}{W'}\right\}} = 0.331$$

$$\delta^2(\hat{\alpha}') = \frac{1}{\Sigma nW'} = 0.049$$

$$\delta^2(\hat{\beta}) = \frac{1}{n\{W'x^2 - \frac{(W'x)^2}{W'}\}} = 0.0006$$

$$\delta^2(C) = 1/(\hat{\beta})^2\{\delta^2(\hat{\alpha}) + (C-\bar{x})^2 x \delta^2(\hat{\beta}) = 0.367$$

Confidence intervals for

$\hat{\alpha}$ = $\hat{\alpha} \pm t_\alpha \sqrt{\delta^2(\hat{\alpha})}$

$-2.645 \pm 1.96\sqrt{0.331}$ -1.51, -3.77

$\hat{\beta}$ = $\hat{\beta} \pm t_\alpha \sqrt{\delta^2(\hat{\beta})}$

$0.37 \pm 1.96\sqrt{0.006}$ 0.22, 0.52

C = $C \pm t_\alpha \sqrt{\delta^2(C)}$

$7.16 \pm 1.96\sqrt{0.367}$ 5.97, 8.34

Lethal Doses

$$LD_{50} = \frac{\ln 1-\hat{\alpha}}{\hat{\beta}} = 7.16$$

$$LD_{80} = \frac{\ln 4-\hat{\alpha}}{\hat{\beta}} = 10.91$$

$$LD_{90} = \frac{\ln 9-\hat{\alpha}}{\hat{\beta}} = 13.10$$

Standard Errors for

$$LD_{50} = 1/(\hat{\beta})^2 \{\delta^2(\hat{\alpha}') + (LD_{50}-\bar{x})^2 x \delta^2(\hat{\beta})\} = 0.367$$

$$LD_{80} = 1/(\hat{\beta})^2 \{\delta^2(\hat{\alpha}) + (LD_{80}-\bar{x})^2 x \delta^2(\hat{\beta})\} = 1.150$$

$$LD_{90} = 1/(\hat{\beta})^2 \{\delta^2(\hat{\alpha}') + (LD_{90}-\bar{x})^2 x \delta^2(\hat{\beta})\} = 2.177$$

Confidence Intervals for

$$LD_{50} = LD_{50} \pm t_{\alpha}\sqrt{\delta^2(LD_{50})}$$

$7.16 \pm 1.96\sqrt{0.367}$ 5.97, 8.34

$$LD_{80} = LD_{80} \pm t_{\alpha}\sqrt{\delta^2(LD_{80})}$$

$10.91 \pm 1.96\sqrt{1.150}$ 8.80, 13.01

$$LD_{90} = LD_{90} \pm t_{\alpha}\sqrt{\delta^2(LD_{90})}$$

$13.10 \pm 1.96\sqrt{2.177}$ 10.20, 15.99

Appendix 23

Logit χ^2

x	W'	l	l^	$(l-l^)^2$	$W'(l-l^)^2$
2	0.1275	-1.7346	-1.9040	0.0286	0.0036
4	0.1875	-1.0986	-1.1580	0.0035	0.0006
6	0.2275	-0.6190	-0.4120	0.0428	0.0097
8	0.2400	0.4054	0.3320	0.0053	0.0012
10	0.2100	0.8472	1.0780	0.0532	0.0118
12	0.0243	3.6635	1.8230	3.3874	0.0823
					0.1092

$$\chi^2_4 = n\{W'(l-l^)^2\} = 2.184 \text{ N S } P < 0.05$$

The $\chi^2_4 = 2.184$, which is not significant as the significance at 5% level the $\chi^2_4 > 9.49$

Appendix 24

Logistic Curve $\frac{1}{1+e^{-(\alpha\hat{}+\beta\hat{}x)}} + \frac{1}{1+e^{-1\hat{}}} = P$

1	e^{-1}	$1+e^{-1}$	$\frac{1}{1+e^{-1}}$	Y=20xP
2.276	9.737	10.737	0.093130	1.8626
1.904	6.712	7.712	0.129656	2.5931
1.531	4.622	5.622	0.177847	3.5569
1.158	3.183	4.183	0.239030	4.7806
0.785	2.192	3.192	0.313243	6.2648
0.412	1.509	2.509	0.398432	7.9686
0.040	1.408	2.408	0.490000	9.8000
-0.332	0.717	1.717	0.582245	11.6449
-0.705	0.494	1.494	0.669295	13.3859
-1.078	0.340	1.340	0.746115	14.9223
-1.450	0.234	1.234	0.809998	16.1999
-1.823	0.165	1.165	0.860925	17.2185

Appendix 25

Permethrin (5% a.i.) Topical

"n"	"x"	"r"	Mortality %	P=r/n	q=1-P	P/q	l=lnP/q
10	"C"	-	-	-	-	-	-
10	5	0.5	5	0.05	0.95	0.0526	-2.9444
10	8	1.0	10	0.10	0.90	0.1111	-2.1972
10	10	5.0	50	0.50	0.50	1.000	0
10	12	6.0	60	0.60	0.40	1.5000	0.4054
10	20	9.0	90	0.90	0.10	9.000	2.1972
10	22	9.5	95	0.95	0.05	19.000	2.9444

Appendix 26

W'=Pq	W'x	Wx^2	W'l	W'xl
0.0475	0.2375	1.1875	-0.1398	-0.6992
0.0900	0.7200	5.7600	-0.1977	-1.5819
0.2500	2.5000	25.000	0	0
0.2400	2.8800	34.5600	0.0972	1.1675
0.0900	1.8000	36.0000	0.1977	3.9549
0.0475	1.0450	22.9900	0.1398	3.0768
0.765̇0	9.1825	125.4975	0.0972	5.9181

Since n = 10

nW' = 7.650, nW'x = 91.825, $nW'x^2$ = 1254.975, nW'l=0.972,

nW'xl = 59.81

$$\bar{x} = \frac{\Sigma nWx}{\Sigma nW'} = 12.000$$

$$\bar{l} = \frac{\Sigma nW'l}{\Sigma nW'} = 0.127$$

$$\hat{\beta} = \frac{\Sigma nW'\Sigma nWxl - \Sigma nW'x\Sigma nW'l}{\Sigma nW'\Sigma nW'x^2 - \Sigma(nW'x)^2}$$

$$\hat{\alpha} = 1 - \hat{\beta}\bar{x} = 0.310$$

$$C = \frac{-\hat{\alpha}}{\hat{\beta}} = 11.590$$

Standard errors and confidence intervals

$$\delta^2(\hat{\alpha}) = \frac{1}{\Sigma nW'} + \frac{\bar{x}^2}{n\{W'x^2 - \frac{(W'x)^2}{W'}\}}$$

$$= 1.076$$

$$\delta^2(\alpha\hat{}) = \frac{1}{n\{W\}} = 0.130$$

$$\delta^2(\beta\hat{}) = \frac{1}{n\{W'x^2 - \frac{(W'x)^2}{W}} = 0.006$$

$$\delta^2(C) = 1/(\beta\hat{})^2\{\delta^2(\alpha\hat{}\,') + (C-\bar{x}) \times \delta^2(\beta\hat{})\} = 1.363$$

Confidence intervals for

$$\alpha\hat{} = \alpha\hat{} \pm t_\alpha\sqrt{\delta^2(\alpha\hat{})}$$

$$= -3.600 \pm 1.96\sqrt{1.076} \qquad -1.56, -5.63$$

$$\beta\hat{} = \beta\hat{} \pm t_\alpha\sqrt{\delta^2(\beta\hat{})}$$

$$= 0.310 \pm 1.96\sqrt{0.006} \qquad 0.16, 0.46$$

$$C = C \pm t_\alpha\sqrt{\delta^2(C)}$$

$$11.59 \pm 1.96\sqrt{1.363} \qquad 9.30, 13.88$$

Lethal doses

$$LD_{50} = \frac{\ln 1-\alpha\hat{}}{\beta\hat{}} = 11.59$$

$$LD_{80} = \frac{\ln 4-\alpha\hat{}}{\beta\hat{}} = 16.05$$

$$LD_{90} = \frac{\ln 9-\alpha\hat{}}{\beta\hat{}} = 18.66$$

Standard errors for

$$LD_{50} = 1/(\beta\hat{})^2\{\delta^2(\alpha\hat{}\,') + (LD_{50}-\bar{x})^2 \times \delta^2(\beta\hat{})\} = 1.363$$

$$LD_{80} = 1/(\beta\hat{})^2\{\delta^2(\alpha\hat{}\,') + (LD_{80}-\bar{x})^2 \times \delta^2(\beta\hat{})\} = 2.38$$

$$LD_{90} = 1/(\beta\hat{})^2\{\delta^2(\alpha\hat{}\,') + (LD_{90}-\bar{x})^2 \times \delta^2(\beta\hat{})\} = 4.12$$

Confidence intervals for

$$LD_{50} = LD_{50} \pm t_{\alpha}\sqrt{\delta^2(LD_{50})}$$

$$11.59 \pm 1.96\sqrt{1.363} \qquad 9.30,\ 13.88$$

$$LD_{80} = 16.05 \pm 1.96\sqrt{2.38} \qquad 13.02,\ 19.07$$

$$LD_{90} = LD_{90} \pm t\sqrt{\delta^2(LD_{90})}$$

$$18.66 \pm 1.96\sqrt{4.12} \qquad 14.68,\ 22.63$$

Appendix 27

Logit χ^2

x	W'	l	l^	$(l-l^)^2$	$W'(l-l^)^2$
5	0.0475	-2.9444	-2.0471	0.8051	0.0382
8	0.0900	-2.1972	-1.1153	1.1705	0.1053
10	0.2500	0	-0.4941	0.2441	0.0610
12	0.2400	0.4054	0.1271	0.0774	0.0185
20	0.0900	2.1972	2.6119	0.1719	0.0154
22	0.0475	2.9444	3.2331	0.0833	0.0039
					0.2426

$$\chi^2_4 = n\{W'(l-l^)^2\} = 2.426 \text{ NS} \quad P < 0.05$$

The values of $\chi^2_4 = 2.426$, which is not significant as $\chi^2_4 > 9.59$ at 5% level.

Appendix 28

Logistic Curve

$$\frac{1}{1+e^{-(\alpha\hat{}+\beta\hat{}x)}} = \frac{1}{1+e^{-1\hat{}}} = P$$

-1	e^{-1}	$1+e^{-1}$	$\frac{1}{1+e^{-1}}$	Y=10xP
2.0471	7.7454	8.7454	0.1143	1.143
1.7365	5.6774	6.6774	0.1497	1.497
1.4259	4.1616	5.1616	0.1937	1.937
1.1153	3.0504	4.0504	0.2468	2.468
0.8047	2.2360	3.2360	0.3090	3.090
0.4941	1.6390	2.6390	0.3789	3.789
0.1835	1.2014	2.20.14	0.4542	4.542
-0.1271	0.8806	1.8806	0.5317	5.317
-0.4377	0.6455	1.6455	0.6077	6.077
-0.7483	0.4731	1.4731	0.6788	6.788
-1.0589	0.3468	1.3468	0.7424	7.424
-1.3695	0.2542	1.2542	0.7972	7.972
-1.6801	0.1863	1.1863	0.8429	8.429
-1.9907	1.1365	1.1365	0.8798	8.798
-2.3013	0.100L	1.1001	0.9089	9.089
-2.6119	0.0733	1.0733	0.9316	9.316
-2.9225	0.0537	1.0537	0.9489	9.489
-3.2331	0.0394	1.0394	0.9626	9.626

Appendix 29

Permethrin (5% a.i.) Stomach poison /grass

"n"	"x"	"r"	Mortality %	P=r/n	q=1-P	P/q	l=lnP/q
10	"C"	-	-	-	-	-	-
10	20	0.5	5	0.05	0.95	0.05263	-2.9444
10	22	2.0	20	0.20	0.80	0.2500	-1.3862
10	24	3.0	30	0.30	0.70	0.4285	-0.8472
10	28	6.0	60	0.60	0.40	1.5000	0.4054
10	30	7.0	70	0.70	0.30	2.3333	0.8472
10	34	9.0	90	0.90	0.10	9.000	2.1972
10	40	9.5	95	0.95	0.05	19.000	2.9444

Appendix 30

W'=Pq	W'x	$W'x^2$	W'l	W'xl
0.0475	0.9500	19.000	-0.1398	-2.7971
0.1600	3.5200	77.440	-0.2217	-4.8794
0.2100	5.0400	120.960	-0.1779	-4.2698
0.2400	6.720	188.160	0.0972	2.7242
0.2100	6.300	189.000	0.1779	5.3373
0.0900	3.060	104.040	0.1977	6.7234
0.0475	1.900	76.000	0.1398	5.5943
1.005	27.490	774.600	0.0732	8.4329

Since n = 10

$nW' = 10.05$, $nW'x = 274.90$, $nWx^2 = 7746.00$, $nWl = 0.732$ $nWxl = 84.329$

$$\bar{x} = \frac{\Sigma nW'x}{\Sigma nW'} = 27.35$$

$$\bar{l} = \frac{\Sigma nWl}{\Sigma nW'} = 0.073$$

$$\hat{\beta} = \frac{\Sigma nW'x \Sigma nW'xl - \Sigma nW'x \times \Sigma nWl}{\Sigma nW' \times \Sigma nW'x^2 - \{\Sigma nW'x)^2\}} = 0.28$$

$$\hat{\alpha} = \bar{l} - \hat{\beta}\bar{x} = -7.59$$

$$C = \frac{-\hat{\alpha}}{\hat{\beta}} = 27.10$$

Standard errors and confidence intervals for

$$\hat{\alpha} = \frac{1}{\Sigma nW} + \frac{\bar{x}^2}{n\{W'x^2 - \frac{(W'x)^2}{W'}\}} = 3.400$$

$$\hat{\alpha}' = \frac{1}{n\{W'\}} = 0.099$$

$$\delta^2(\hat{\beta}) = \frac{1}{n\{W'x^2 - \frac{(W'x)^2}{W'}} = 0.004$$

$$\delta^2(C) = 1/(\hat{\beta})^2\{\delta^2(\hat{\alpha}') + (C-\bar{x})^2 \times \delta^2(\hat{\beta})\} = 1.24$$

Confidence intervals for

$$\hat{\alpha} = \hat{\alpha} \pm t_\alpha\sqrt{\delta^2(\hat{\alpha})}$$

$-7.59 \pm 1.96\sqrt{3.400}$ $\quad$ $-3.98, -11.20$

$$\hat{\beta} = \hat{\beta} \pm t_\alpha\sqrt{\delta^2(\hat{\beta})}$$

$0.28 \pm 1.96\sqrt{0.004}$ $\quad$ $0.16, 0.40$

$$C = C \pm t_\alpha\sqrt{\delta^2(C)}$$

$27.10 \pm 1.96\sqrt{1.24}$ $\quad$ $24.92, 29.28$

Lethal Doses

$$LD_{50} = \frac{\ln 1-\hat{\alpha}}{\hat{\beta}} = 27.10$$

$$LD_{80} = \frac{\ln 4 -\hat{\alpha}}{\hat{\beta}} = 32.06$$

$$LD_{90} = \frac{\ln 9-\hat{\alpha}}{\hat{\beta}} = 34.95$$

Standard errors for

$$LD_{50} = 1/(\hat{\beta})^2\{\delta^2(\hat{\alpha}') + (LD_{50} - \bar{x})^2 \times \delta^2(\hat{\beta})\} = 1.24$$

$$LD_{80} = 1/(\hat{\beta})^2\{\delta^2(\hat{\alpha}') + (LD_{80} - \bar{x})^2 \times \delta^2(\hat{\beta})\} = 2.39$$

$$LD_{90} = 1/(\hat{\beta})^2\{\delta^2(\hat{\alpha}') + (LD_{90} - \bar{x})^2 \times \delta^2(\hat{\beta})\} = 4.20$$

Confidence intervals for

$LD_{50} = LD_{50} \pm t_{\alpha}\sqrt{\delta^2(LD_{50})}$

27.10 1.96 1.24 24.92, 29.28

$LD_{80} = LD_{80} \pm t_{\alpha}\sqrt{\delta^2(LD_{80})}$

$32.06 \pm 1.96\sqrt{2.39}$ 29.02, 35.09

$LD_{90} = LD_{90} \pm t_{\alpha}\sqrt{\delta^2(LD_{90})}$

$34.95 \pm 1.96\sqrt{4.20}$ 30.93, 38.96

Appendix 31

Logit χ^2

x	W'	l	l^	$(l-l^)^2$	$W(l-l^)^2$
20	0.0475	-2.944	-2.005	0.881	0.0418
22	0.1600	-1.386	-1.437	0.002	0.0004
24	0.2100	-0.847	-0.870	0.0005	0.0001
28	0.2400	0.405	0.264	0.019	0.0047
30	0.2100	0.847	0.831	0.0002	0.00005
34	0.0900	2.197	1.966	0.0534	0.0048
40	0.0475	2.944	3.669	0.5250	0.0249
					0.0767

$$\chi^2_5 \quad n\{W'(1-l\ \hat{})^2\} = 0.767 \text{ NS} \quad P < 0.05$$

The $\chi^2_5 = 0.767$ is not significant, as the significance at 5% level, the $\chi^2 > 11.07$

Appendix 32

Logistic curve

$$\frac{1}{1+e^{-(\alpha\hat{}+\beta\hat{}x)}} = \frac{}{1+e^{-1\hat{}}} = P$$

$-1\hat{}$	$e^{-1\hat{}}$	$1+e^{-1\hat{}}$	$\frac{1}{1+e^{-1\hat{}}}$	Y=10xP
2.005	7.4260	8.4260	0.1186	1.186
1.721	5.5901	6.5901	0.1517	1.517
1.437	4.2080	5.2080	0.1920	1.920
1.154	3.1708	4.1708	0.2397	2.397
0.870	2.3869	3.3869	0.2952	2.952
0.586	1.7967	2.7967	0.3575	3.575
0.302	1.3525	2.3525	0.4250	4.250
0.019	1.0191	2.0191	0.4952	4.952
-0.264	0.7679	1.7679	0.5656	5.656
-0.548	0.5781	1.5781	0.6336	6.336
-0.831	0.4356	1.4356	0.6965	6.965
-1.115	0.3279	1.3279	0.7530	7.530
-1.399	0.2468	1.2468	0.8020	8.020
-1.683	0.1858	1.1858	0.8433	8.433
-1.966	0.1400	1.1400	0.8771	8.771
-2.250	0.1053	1.1053	0.9046	9.046
-2.534	0.0793	1.0793	0.9264	9.264
-2.810	0.0602	1.0602	0.9432	9.432
-3.101	0.4500	1.4500	0.9569	9.569
-3.385	0.0338	1.0338	0.9672	9.672
-3.669	0.0255	1.0255	0.9751	9.751

Appendix 33

Permethrin (5% a.i.) Stomach poison/tissue paper

"n"	"x"	"r"	P=r/n	q=1-P	P/q	l=lnP/q
10	"C"	-	-	-	-	-
10	26	0.5	0.05	0.95	0.052631	-2.9444
10	28	1.0	0.10	0.90	0.111111	-2.1972
10	30	2.0	0.20	0.80	0.250000	-1.3862
10	32	2.0	0.20	0.80	0.250000	-1.3862
10	34	3.0	0.30	0.70	0.428571	-0.8472
10	36	4.0	0.40	0.60	0.666666	-0.4054
10	38	4.0	0.40	0.60	0.666666	-0.4054
10	40	6.0	0.60	0.40	1.500000	0.4054
10	42	7.0	0.70	0.30	2.333333	0.8472
10	44	7.0	0.70	0.30	2.333333	0.8472
10	46	8.0	0.80	0.20	4.000000	1.3862
10	48	9.5	0.95	0.05	19.000000	2.9444

Appendix 34

W'=Pq	W'x	$W'x^2$	W'l	W'xl
0.0475	1.235	32.110	-0.1398	-3.6363
0.0900	2.520	70.560	-0.1977	-5.5369
0.1600	4.800	144.000	-0.2217	-6.6537
0.1600	5.120	163.840	-0.2217	-7.0973
0.2100	7.140	242.760	-0.1779	-6.0490
0.2400	8.640	311.040	-0.0972	-3.5026
0.2400	9.120	346.560	-0.0972	-3.6972
0.2400	9.600	384.000	0.0972	3.8918
0.2100	8.820	370.440	0.1779	7.4723
0.2100	9.240	406.560	0.1779	7.8281
0.1600	7.360	338.560	0.2217	10.2024
0.0475	2.280	109.440	0.1398	6.7132
2.015	75.875	2919.870	-0.3387	0.0652

Since n = 10

nW' = 20.15, nW'x = 758.75, $nW'x^2$ = 29198.70, nWl = -3.387, nW'xl = 0.652

$$\bar{x} = \frac{\Sigma nWx}{\Sigma nW'} = 37.65$$

$$\bar{1} = \frac{\Sigma nW'1}{\Sigma nW'} = -0.168$$

$$\hat{\beta} = \frac{\Sigma nW' \times \Sigma nWl - \Sigma nWx \times \Sigma nWl}{\Sigma nW' \times \Sigma nW'x^2 - (nW'x)^2}$$

$$\hat{\alpha} = \bar{1} - \hat{\beta}\bar{x} = -7.287 = 0.198$$

$$C = \frac{-\hat{\alpha}}{\hat{\beta}} = 36.80$$

Standard errors and confidence intervals

$$\delta^2(\alpha\hat{}) = \frac{1}{\Sigma nW'} + \frac{\bar{x}^2}{n\{W'x^2 - \frac{(Wx)^2}{W'}\}} = 0.109$$

$$\delta^2(\alpha\hat{}') = \frac{1}{n\ W'} = 0.050$$

$$\delta^2(\beta\hat{}) = \frac{1}{n\{W'x^2 - \frac{(W'x)^2}{W'}\}} = 0.0016$$

$$\delta^2(C) = 1/(\beta\hat{})^2\{\delta^2(\alpha\hat{}') + (C-\bar{x})^2 \times \delta^2(\beta\hat{})\} = 1.30$$

Confidence intervals for

$\alpha\hat{} = \alpha\hat{} \pm t_\alpha\sqrt{\delta^2(\alpha\hat{})}$

$-7.287 \pm 1.96\sqrt{0.109}$ -6.64, -7.93

$\beta\hat{} = \beta\hat{} \pm t_\alpha\sqrt{\delta^2(\beta\hat{})}$

$0.198 \pm 1.96\sqrt{0.0016}$ 0.12, 0.28

$C = C \pm t_\alpha\sqrt{\delta^2(C)}$

$36.80 \pm 1.96\sqrt{1.30}$ 34.56, 39.03

Lethal Doses

$$LD_{50} = \frac{\ln\ 1-\alpha\hat{}}{\beta\hat{}} = 36.80$$

$$LD_{80} = \frac{\ln\ 4-\alpha\hat{}}{\beta\hat{}} = 43.80$$

$$LD_{90} = \frac{\ln\ 9-\alpha\hat{}}{\beta\hat{}} = 47.90$$

Standard errors for

$$LD_{50} = 1/(\hat{\beta})^2\{\delta^2(\hat{\alpha}') + (LD_{50} - \bar{x})^2 \times \delta^2(\hat{\beta})\} = 1.30$$

$$LD_{80} = 1/(\hat{\beta})^2\{\delta^2(\hat{\alpha}') + (LD_{80} - \bar{x})^2 \times \delta^2(\hat{\beta})\} = 2.81$$

$$LD_{90} = 1/(\hat{\beta})^2\{\sigma^2(\hat{\alpha}) + (LD_{90} - \bar{x})^2 \times \delta^2(\hat{\beta})\} = 5.56$$

Confidence intervals for

$$LD_{50} = LD_{50} \pm t_{\alpha}\sqrt{\delta^2(LD_{50})}$$

$$36.80 \pm 1.96\sqrt{1.30} \qquad 34.56, 39.03$$

$$LD_{80} = LD_{80} \pm t_{\alpha}\sqrt{\delta^2(LD_{80})}$$

$$43.80 \pm 1.96\sqrt{2.81} \qquad 40.51, 47.08$$

$$LD_{90} = LD_{90} \pm t_{\alpha}\sqrt{\delta^2(LD_{90})}$$

$$47.90 \pm 1.96\sqrt{5.56} \qquad 43.27, 52.52$$

Appendix 35

Logit χ^2

x	W'	l	l^	$(l-l^)^2$	$W'(l-l^)^2$
26	0.0475	-2.944	-2.467	0.2275	0.0108
28	0.0900	-2.197	-2.077	0.0144	0.0013
30	0.1600	-1.386	-1.681	0.0870	0.0139
32	0.1600	-1.386	-1.285	0.0102	0.0016
34	0.2100	-0.847	-0.889	0.0017	0.0004
36	0.2400	-0.405	-0.494	0.0079	0.0019
38	0.2400	-0.405	-0.098	0.0942	0.0226
40	0.2400	-0.405	0.297	0.4928	0.1182
42	0.2100	0.847	1.088	0.0580	0.0129
44	0.2100	0.847	1.088	0.0580	0.0129
46	0.1600	1.386	1.484	0.0096	0.0015
48	0.0475	2.944	1.879	1.1342	0.0538
					0.2439

$$\chi^2_{10} = n\{ W'(l-l^)^2\} = 2.439 \text{ NS } P < 0.05$$

The value of χ^2_{10} = 2.439, which is not significant as the significance at 5% level the χ^2_{10} > 18.31.

Appendix 36

Logistic Curve

$$\frac{1}{1+e^{-(\alpha\hat{}+\beta\hat{}x)}} = \frac{1}{1+e^{-1\hat{}}} = P$$

$-1\hat{}$	$e^{-1\hat{}}$	$1+e^{-1\hat{}}$	$\frac{1}{1+e^{-1\hat{}}}$	Y=10xP
2.472	11.846	12.846	0.0778	0.778
2.247	9.718	10.718	0.0932	0.932
2.077	7.980	8.980	0.1113	1.113
1.879	6.546	7.546	0.1325	1.325
1.681	5.370	6.370	0.1569	1.569
1.483	4.406	5.406	0.1849	1.849
1.285	3.614	4.614	0.2167	2.167
1.087	2.965	3.965	0.2521	2.521
0.889	2.432	3.432	0.2913	2.913
0.692	1.997	2.997	0.3335	3.335
0.494	1.638	2.638	0.3789	3.789
0.296	1.344	2.344	0.4265	4.265
0.098	1.102	2.102	0.4755	4.755
-0.092	0.912	1.912	0.5229	5.229
-0.297	0.743	1.743	0.5737	5.737
-0.494	0.610	1.610	0.6210	6.210
-0.692	0.500	1.500	0.6664	6.664
-0.890	0.410	1.410	0.7088	7.088
-1.088	0.336	1.336	0.7480	7.480
-1.286	0.276	1.276	0.7834	7.834
-1.484	0.226	1.226	0.8151	8.151
-1.682	0.186	1.186	0.8431	8.431
-1.879	0.152	1.152	0.8674	8.674